MALARIAL DISEASES

INTRODUCTION

TO THE STUDY OF

MALARIAL DISEASES

BY

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LONDON

REBMAN, LIMITED

129, SHAFTESBURY AVENUE, CAMBRIDGE CIRCUS, W.C.

1903

AUTHOR'S PREFACE

This little book does not offer much that is new to the student of malaria. Novelty, however, is not its main purpose. The intention of the author was, rather, to provide the ship's surgeon and the colonial surgeon, often thrown entirely on their own resources, with a book in which they could find advice, without this advice occupying too great a space. I have therefore endeavoured to be as concise as possible, and have described with completeness only the methods of investigation, and the difficulties and errors that may arise during the search for malarial parasites. Other errors of observation and the means of avoiding them have also been discussed, and I hope, therefore, that my book may succeed in facilitating the recognition, management, and prevention of malarial fever.

The photographic plates were prepared by that master of micro-photography, Professor Zettnow.

REINHOLD RUGE.

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MALARIA

DISTRIBUTION AND HISTORY OF MALARIA.

The purpose of this little work is to assist the ship's surgeon or the colonial surgeon who has to undertake unaided the diagnosis, treatment, and prevention of malarial fever. I shall, therefore, consider but briefly the distribution and history of the disease.

Malarial fever is found all over the world, in an area extending from 40° south latitude to 60° north latitude. At certain times of the year the ship's surgeon encounters malarial fever in the majority of tropical and subtropical harbours. Low-lying, flat, swampy stretches of coast are especially the seats of malarial fever; but certain elevated rocky islands—the Cape Verd Islands, for instance—are not exempt from it. The diffusion of malarial fever being thus extensive, it is not surprising that from the earliest times the disease has aroused the greatest interest, not only in physicians, but also in the laity, and that the literature of the subject is so enormous that no single man can now hope to master it.

The history of malarial fever may be divided into three periods.

First Period.—This extends from the time of Hippocrates down to the year 1640, when the bark of the cinchona-tree was brought for the first time to Europe by the Countess Cinchon, wife of the Viceroy of Peru, the Countess by its use having been cured of an intermittent fever. An enormous advance was thus effected, for although from time to time during the following centuries this drug fell into disrepute, either because it was employed in the treatment of fevers which were not of a malarial nature, or because, in true intermittents, it was administered at an improper time, so that its effects were unsatisfactory, nevertheless, it was already of inestimable value, for physicians were no longer so powerless as they had previously been in the treatment of intermittent fever.

Second Period.—This extends from 1640 to 1880, the year in which Laveran discovered the parasites of malaria. On November 6, 1880, A. Laveran, then a surgeon in the French military service, stationed at Constantine, Algeria, saw for the first time the malarial parasites in the blood of a patient suffering from intermittent fever. At a later date, Marchiafava and Celli endeavoured to dispute Laveran's claim to priority in this discovery, notwithstanding the fact that in 1884, at the Copenhagen Congress, they still maintained that the malarial parasites were morbid changes in the erythrocytes. They contended that in Rome in the year 1882 Laveran demonstrated to them nothing more than pigmented bodies, such as had previously been observed by others, which were certainly not parasitic in nature.[1] It would be futile to enter further into this dispute concerning priority, since it is definitely established that Laveran was the first to see the malarial parasites, even if some of the forms of these parasites were erroneously described by him.*

Third Period.—This comprises the latest researches, from the year 1880 down to the present time, including the discovery of the life-history of the malarial parasites in the human blood (endogenous cycle) by Golgi (1885), the transmissibility of these parasites by mosquito bites (Ross, 1897 and 1898), and the work of R. Koch and many other observers.

I. ETIOLOGY: MALARIAL PARASITES AND MOSQUITOES.

Intermittent fevers are produced by parasites which belong to the division of protozoa, and are closely allied to the coccidia, but should perhaps be placed in an independent order. The differences between the malarial parasites and the coccidia have been summarized by Koch in the following terms[2]:

'They' (the malarial parasites) 'are at the commencement of their existence parasites which live in the interior or on the surface of the erythrocytes, and, as the formation of pigment in their protoplasm proves, they are nourished on the constituents of these corpuscles. The coccidia, on the other hand, are parasites of epithelial cells, and contain no pigment.

* Laveran, in his *Traité du Paludisme*, 1898, p. 43, writes as follows: 'All this did not prevent M. Marchiafava from trying to claim for himself a considerable share in the discovery of the hæmatozoa of paludisme. I will not dwell on my own claims, for the matter may now be regarded as finally settled.'

'In the endogenous cycle—that is to say, in the intrahuman phase of their development—the malarial parasites increase in number by simple fission, without the formation of an investing membrane. The young germs are not crescent-shaped, but are ovoid or spherical, and they are few in number. The coccidia, on the other hand, have an investing membrane (capsule), and, when undergoing division, break up into a large number of crescent-shaped spores.'

Especially characteristic of the malarial parasites is the first stage of their exogenous development (development in the terminal host).* 'The parasites pass out of the erythrocytes, and assume a spherical form. If the parasites thus set free in the blood are stained by Romanowsky's method, two different forms may be distinguished. One of these has a large and compact chromatin body, while its protoplasm stains but faintly, whereas in the other form of parasite the protoplasm stains more deeply, and there is less chromatin. In the case of the first-named form, the chromatin passes out of the body of the parasite in the shape of filaments, and these filaments, which are formed in limited numbers only, are spermatozoa, which serve to fertilize the parasites of the second kind—those containing but little chromatin. These latter, after fertilization, develop into vermicular bodies.'

The characters just described are peculiar to the true malarial parasites, to which order the malarial parasites of human blood belong. These latter form two groups:

1. The large parasites.

(*a*) The parasite of tertian fever (*Hæmamœba vivax*).

(*b*) The parasite of quartan fever (*Hæmamœba malariæ vel Laverani*).

2. The small or ring-shaped parasites (parasites of tropical fever, of summer-autumn fever, crescent-forming parasites—*Hæmomenas Laverania*).†

All the human malarial parasites have two alternate generations:

* According to zoologists, man is to be regarded as the intermediate host of the malarial parasites, because it is in the human blood that the asexual reproduction of these bodies occurs. The mosquito, on the other hand, is to be regarded as the terminal host, because it is in the mosquito (anopheles) that the sexual reproduction of the human malarial parasites takes place.

† Translator's Note: Certain authors describe two other species of small crescent-forming parasites, each with a twenty-four hour cycle of endogenous development, viz., the pigmented quotidian parasite and the unpigmented quotidian parasite. Dr. Ruge, however, disregards these, and considers the three species mentioned in the above classification to be the only human malarial parasites of which we have a definite knowledge.

(*a*) An asexual generation, in which asexual reproduction occurs in the human blood (endogenous cycle); and

(*b*) A sexual generation, in which sexual reproduction occurs within the bodies of mosquitoes belonging to the genus anopheles (exogenous cycle).

A. Life-history of the Malarial Parasites within the Human Body (Endogenous Cycle).

1. The Large Parasites.

(*a*) *The Parasite of Tertian Fever.*

On examining the blood of a patient suffering from tertian fever, either when the fever is at its height or during its decline, we observe the young forms of the tertian parasite (erroneously called 'spores') within the substance or adhering to the surface of the erythrocytes. In preparations stained with methylene-blue, they appear as small blue, ovoid bodies, the diameter of which is about one-sixth that of an erythrocyte, stained more deeply at the periphery than in the middle. We also find parasites in the form of small blue rings, of a diameter of from one-third to one-half of that of an erythrocyte. These rings are in many cases made up of two halves of different thickness. One of these halves appears as fine as a hair, and has a nodal swelling in the middle; whilst the other half of the ring, opposite to that containing the nodal swelling, is thickened and crescentic in shape. These rings are called small tertian rings. In other cases, however, the ring may be of almost equal thickness throughout, and the nodal swelling, which has led to the comparison of these rings to a signet ring, may be but faintly indicated. In some preparations the rings may appear distorted, so as to have an oval form, and when we see the crescentic thickening of a ring in an isolated optical section, we may have produced the appearance of a tadpole. Finally, a ring may be torn, or its sides may be pressed upon, so that it assumes the appearance of a paper kite.*

Twenty-four hours after the paroxysm of fever the ring has increased considerably in size, all its parts are greatly thickened, and it contains a great number of minute buff to dark-brown short rods and fine granules of pigment. It now occupies from one-third to one-half of the erythrocyte, which has itself increased to the extent of from one and a half times to twice its original diameter, and has become much

* The young parasites, lying within the erythrocytes (endoglobular parasites), have also been called amœbulæ or myxopods.

paler in tint. These rings are known as large tertian rings. The parasite may, however, assume other forms. The second form, which is almost as common as the large tertian ring, and, indeed, in many preparations is even commoner, may best be compared to an amœba which has undergone coagulation at the moment when all its pseudopodia were fully extended. Parasites of this type have at times the most grotesque shapes imaginable. Less frequently the parasite appears as a blue, more or less accurately shaped disc. Finally, thirty-six hours after the access of fever, the rings have entirely disappeared, and the grotesque amœboid forms are rarely to be seen. The parasites, which now occupy three-fourths of the enlarged erythrocytes, appear as compact blue discs, throughout the substance of which pigment is abundantly distributed. The infected erythrocytes are nearly all enlarged to double their original size, and have become very much paler than normal.

Just before the new paroxysm, the parasites, being now more than forty-four hours old, occupy more or less completely the whole substance of the infected erythrocytes, and have the appearance of oval or polygonal blue discs. The pigment has either agglomerated into a mass in the centre of the parasite, or is distributed in irregular lines, radiating from the centre like the spokes of a wheel. Finally, at the onset of the paroxysm, the parasites present themselves as round or oval bodies with a crenulated border, the mean diameter of which is about one and a half times that of an erythrocyte. The pigment is agglomerated in masses, either at the centre of the parasite or near its margin. At this time, moreover, the parasites show distinct indications of commencing division, as is evident in preparations stained simply with methylene-blue. The parasite is seen to consist of from fifteen to twenty-five ovoid parts, these being the young parasites, and the whole body resembles in appearance a mulberry or a raspberry (morula-form). These bodies, which are now either free in the blood, or surrounded merely by a fine ring representing the remains of the erythrocyte, are known as fission-forms, sporulation-forms, or sporocytes. On the rupture of the envelope formed by the remains of the erythrocyte, the young parasites are set free in the blood, and gain entrance into fresh erythrocytes, while the residual masses of pigment are taken up by the leucocytes.

It must not, however, be imagined that the just described life-history of the tertian parasite proceeds with mathematical regularity, and that when the fever is at its height, in a simple tertian infection, we shall find nothing but the youngest forms of the parasite (spores).

The parasites do not all undergo fission* (sporulation) simultaneously. A few of them divide during the last hours before the paroxysm, the great majority during the onset, and a few during the decline of the fever. Hence during the height of the paroxysm of fever we may find on the one hand isolated, belated sporocytes, and on the other hand parasites which have already assumed the ringed form. Again, in the later stages of development, the parasites that come under our observation are not all of exactly the same size, but large and small rings may be seen side by side. Later still, at the beginning of the next paroxysm, we find, in addition to numerous fission-forms, parasites in which fission is only just about to begin, and which do not yet completely fill the erythrocytes, and side by side with these we see very young parasites (spores), isolated or in heaps, lying free in the blood.

The forms of the parasite hitherto described are collectively named asexual, febrinogenic, or active forms (schizonts, according to Schaudinn), in contradistinction to the sexual forms, now to be described.

When a patient has undergone two or three paroxysms of fever, we find, in all stages of the fever, certain full-grown parasites, which either fill almost entirely the substance of the greatly enlarged erythrocyte in which they are found, or else are quite free in the blood. Though few in number, these parasites have a very characteristic appearance. They may attain a size double that of a normal erythrocyte, and they may be distinguished by the fact that they are stained a dull-blue colour; while their pigment, which in other parasites that have attained such large dimensions is always aggregated in masses, is in these bodies distributed indifferently throughout their substance. Finally, they present no signs whatever of commencing fission. These forms are known as spheres (gametes);† their significance will be discussed later.

* Translators' Note: With reference to the use of the term 'fission' for the process here described, a leading English authority on malaria has called our attention to the fact that though this word is a literal translation of the German 'theilung,' yet in English biology the significance of the term 'fission' has become limited to division of a cell into *two* parts only. We should speak, therefore, of the 'fragmentation' or 'segmentation' of the malarial parasites, not of the 'fission' of these organisms. We were made aware of this error too late to correct it in the text, and we must content ourselves with asking our readers to substitute mentally 'fragmentation' for 'fission' wherever the latter term is used in this work.

† I must point out here at the outset that the terms 'sphere' and 'gamete' are not fully synonymous. The full-grown, free gametes only are called spheres.

(*b*) *The Parasite of Quartan Fever.*

The life-history of the quartan parasite is in many respects exceedingly similar to that of the tertian parasite. The developmental cycle, however, occupies three days, and the processes of growth are for this reason somewhat slower. Notwithstanding this fact, in the youngest forms of the two parasites, while they still appear as rings, and before the erythrocytes infected by the tertian parasites have undergone enlargement, to distinguish between the two kinds is sometimes impossible, and it is not until twenty-four hours after the onset of the fever that the distinction can be made with certainty. At this stage the quartan parasite appears as a narrow, pigmented, blue band, lying across one of the diameters of the erythrocyte, which is itself neither enlarged nor paler than normal. Forty-eight hours even after the onset of the fever, when the parasite appears as a broad band, occupying three-fourths or more of the erythrocyte, the latter is neither enlarged nor decolourized; nor is this the case even at the commencement of the new paroxysm of fever, although twelve hours earlier than this the parasite has already completely filled the infected erythrocyte. In fact, the diameter of the quartan parasite never exceeds that of the normal erythrocyte, and the erythrocytes infected by this parasite become neither enlarged nor decolourized. Further, in the case of the quartan parasite, we never see the grotesque amœboid forms met with among the half-matured tertian parasites. The development of the quartan parasite is throughout quieter and more regular. The quartan parasites resemble rather irregularly formed, compact, blue flattened bodies, and are richer in pigment than the tertian parasites; or they extend as broad blue bands diametrically across the erythrocytes.*

Finally, when undergoing fission, the quartan parasite forms at most twelve, and more commonly only eight, young parasites (spores). Golgi formerly described as typical a fission-form resembling a daisy, but this is seldom well developed. Frequently the young parasites are similarly arranged to the young tertian parasites. Among the quartan parasites we find, under similar circumstances, forms similar to those which, in the case of the tertian parasites, were described as spheres or gametes. They are distinguished from the tertian spheres by the following characters only: first, that they are never larger

* Ziemann writes: 'In shape the quartan parasites may be rounded, but very often they have an exceedingly characteristic broad ribbon-shape, the ribbon being so placed that it extends across the erythrocyte from one point in the periphery to the corresponding point on the opposite side.'[3]

than a normal erythrocyte; and, secondly, that they are more deeply pigmented than the tertian spheres.

2. The Small Parasites of Tropical Fever.

Essentially different from the parasitic forms just described are the forms exhibited by the parasites found in tropical fevers (malignant tertian, *tertiana gravis*, æstivo-autumnal or summer-autumn fever). In these cases, in the early part of the fastigium, we find minute blue-black rings, of the fineness of a hair, with a sharp contour, as if drawn with a pen. In diameter they are from one-sixth to one-fifth of the diameter of an erythrocyte. In the periphery of the ring we always find a nodal swelling. These rings are known as small tropical rings. While the fever is at its height and when it is nearing its end, we find rings of a medium size (medium-sized tropical rings), with similar characters to the small rings, but of a diameter of one-fourth to one-third of that of an erythrocyte. In many cases these rings have two nodal swellings, which are always opposite to one another. Often, the ring is not completely closed, the parasite then having a horse-shoe form. The medium-sized tropical rings may already show a slight thickening of the half of the ring lying opposite to the nodal swelling, thus indicating a transition to the so-called large tropical rings, which are found during defervescence and in the non-febrile stage. The large tropical rings are distinguished by marked thickening of the half of the ring opposite to the nodal swelling, which thus assumes a crescentic form, while the other half remains as fine as a hair. The large tropical rings have a diameter of one-third to one-half of that of an erythrocyte.

Tropical rings are distinguishable from one another, not only in shape and size, but also in the number in which they appear in the blood. The small tropical rings are, as a rule, found isolated and in small numbers only; the medium-sized rings are found in still scantier numbers; while the large tropical rings come under observation in relatively much greater numbers. Fission-forms (so-called sporulation-forms) of the tropical parasite are very rarely seen in blood drawn from the finger; but they are all the more abundant in the blood of the spleen, the brain, and the red marrow of bones. These fission-forms are small blue discs, occupying from one-half to two-thirds of an erythrocyte, with one or two masses of pigment in their interior. They divide into from fifteen to twenty-five young parasites, which have similar characters to the young tertian parasites previously described, but the young tropical parasites are notably smaller than the young parasites belonging to the two larger varieties. As is the case with the latter, so also the development of the tropical para-

sites does not always take place in the exact and systematic manner just described. Thus it may happen that at the onset of the fresh attack of fever, isolated large tropical rings may still remain as stragglers in the blood. If, now, in such a case the small tropical rings develop earlier or in greater numbers than usual, we then find small rings associated with large rings at the commencement of the attack of fever. We must not, however, conclude from this that there are two generations of parasites in the blood, for, in the further course of the fever, the large rings very rapidly disappear. Similarly, at times, during the fastigium, we may find small and medium-sized rings side by side, and during defervescence medium-sized and large rings, simply because each form proceeds from the one before it in uninterrupted succession. But we may always observe that as soon as the first isolated individuals of the new phase have made their appearance, forms of this character soon occupy the whole field, and those belonging to the previous phase rapidly disappear.

When we come to examine the blood of a patient who has suffered from tropical fever for a considerable period, we find other forms in addition to rings. These are the so-called crescents (gametes), bodies which, as the name implies, have a form resembling that of the crescent moon. The horns of the crescent (poles) are, however, usually rounded off, and stain with methylene-blue more deeply than the centre, where the pigment is found as a deposit of granules arranged in a circle. The length of a crescent is equivalent to from one and a half to two diameters of an erythrocyte. Often crescents are seen to be still contained in an erythrocyte, of which nothing more remains distinguishable beyond a delicate line extending across the concavity of the crescent. In addition to the crescents, spheres are also found resembling in shape, colour, and arrangement of pigment the spheres of the larger varieties of parasites. They are, however, somewhat smaller than these. They are formed out of crescents, which become first spindle-shaped, then ovoid and finally spherical.

Such are the appearances of the malarial parasites when studied in preparations stained with methylene-blue only. By this method the difference between nuclear substance and protoplasm cannot be clearly made out. If we desire a staining reaction by which this difference is rendered visible, we must employ the double-staining method of Romanowsky. The nuclear substance (chromatin) is then stained a brilliant red colour, the protoplasm of the parasites blue, and the erythrocytes pink. This method of staining also allows us to follow closely the process of nuclear division (karyokinesis). I shall therefore proceed to give a description of the intimate structure

of the malarial parasite and of its method of growth, based upon the results obtained by Romanowsky's staining process. I shall take as example the tertian parasite, the development of which can be most easily made out.

Simple methylene-blue staining has already shown us that the young, newly-formed parasite (spore) is ovoid in shape, and that one of its poles is wider, the other narrower. Both these poles are stained blue. Romanowsky's method, however, shows us that the whole of the narrower pole of the young parasite consists of a single sharply-defined, large, oval, brilliant-red granule, on which the wider pole is superimposed as a narrow blue crescent. This red granule is the nuclear substance, the chromatin.*

In the ring-forms, the nodal swelling is stained red—consists, that is, of chromatin—while the rest of the ring is blue—consists of the cell protoplasm. The chromatin is not always found in the form of a granule; it may have the shape of a small rod. It may also lie in the centre of the ring, and apparently disconnected from this. The larger the parasite grows, the larger also is the mass of nuclear chromatin. It then loses its nodular appearance, increases irregularly in width, and divides into two parts. These parts subdivide again and again, until at length we have formed a body containing fifteen or more oval or rod-shaped chromatin segments, each of which is always surrounded by a certain quantity of protoplasm. The fission-form (sporulation-form) is now complete.

Occasionally, especially in half-grown parasites, we can make out an unstained zone, surrounding the chromatin in the form of a ring. This is known as the achromatic zone. It is supposed that this zone represents the nuclear matrix. The division of the chromatin begins in the tertian parasite about twelve hours, in the quartan about twenty-four hours, before the next paroxysm of fever (Ziemann).

In the spheres (gametes) the arrangement of the chromatin is quite different from that found in the forms just described (*cf.* note on p. 6).

Two different kinds of gametes may be distinguished, male and female. In the former the chromatin is present in considerable quantities, aggregated in fine filaments; but in the female gametes it is scanty, and is in the form of very minute granules. Further, while in the male gametes the protoplasm is stained of a light-blue colour, in the female gametes it is stained dark blue. Finally,

* Whereas in the newly-formed parasite the chromatin and the protoplasm are about equal in quantity, in the further development of the parasite it is especially the blue-stained protoplasm that undergoes growth.

even with the use of Romanowsky's method, it is impossible in the case of the gametes to make out any indications of commencing division of the chromatin.

In the parasites of tropical fever Romanowsky's method also shows us in the tropical rings, just as in the tertian rings, that the chromatin corresponds to the nodal swelling, which is stained red, while the ring itself assumes a blue colour. The fission-forms of the tropical parasites stained by Romanowsky's method are analogous to those of the tertian and quartan fevers, with the difference that the young tropical parasites are much smaller than the others.

By means of Romanowsky's staining method we are enabled, not merely to demonstrate chromatin in the crescents and spheres of tropical fevers, but also to show that these bodies are of two different kinds—one containing much chromatin, with protoplasm staining a light-blue colour; the other containing little chromatin, with protoplasm staining a dark-blue colour.

I will now briefly recapitulate the differences we have found to exist between the various species of parasites.

There are certain criteria which establish immediately the differential diagnosis between the three species of parasites. The varying duration of the developmental processes is inapplicable for the purpose of microscopical diagnosis, but the fact that an erythrocyte infected by a tertian parasite is, after sixteen hours, already swollen and partially decolourized leads immediately to the diagnosis of tertian fever. But prior to this enlargement and decolourization of the erythrocytes, the young forms of the parasites of tertian and quartan fever resemble one another so closely that they cannot be distinguished one from another with absolute certainty,* unless, indeed, we find the narrow bands characteristic of the quartan parasite.

It is quite otherwise as regards the young forms of tropical fever. Neither the tertian nor the quartan parasite forms rings of extreme and uniform tenuity, such as we have found to be characteristic of the small and medium-sized tropical parasites; but confusion may arise between small tertian and quartan rings on the one hand, and large tropical rings on the other. These three kinds of rings have such a close resemblance that they cannot be distinguished one from another. All three alike have a crescentic thickening lying opposite to the nodal swelling. Their diameter is about one-third of that of an erythrocyte, and the infected erythrocyte is neither enlarged nor decolourized.

* It may happen that we can find in the blood isolated spheres only, and doubt may then arise whether we have to do with tertian or quartan parasites, for the tertian spheres are sometimes very small, and thus may resemble quartan spheres.

Moreover, Romanowsky's ordinary staining method brings out no difference between them, and the modification of this method, which we shall describe later, differentiates the tertian parasite only, and this not always with certainty. We must therefore search for other means to enable us to establish a diagnosis. Such means are fortunately available. First, in almost all cases of tertian and quartan fever we find in the blood, in addition to the ring-forms, isolated large forms (spheres), which at once establish the diagnosis, since these forms are not found in tropical fever.

On the other hand, there are forms which are found only in tropical fever—namely, crescents and small and medium-sized tropical rings. If, therefore, in addition to rings of a doubtful nature, we find full-grown spheres of the larger parasite, we can make a definite diagnosis of tertian or quartan fever, as the case may be; whereas if we find crescents, or either small or medium-sized tropical rings, the diagnosis of tropical fever is established. Failing these points of distinction, we must fall back on the course and the type of the fever. As we shall see later, the intermittent fevers due to the larger parasites are in their clinical course very different from those due to small parasites, the tropical or summer-autumn fevers.

It must further be observed that mixed infections with small and large parasites occur. Indeed, either tertian or quartan parasites may be found in conjunction with the parasites of tropical fever.*

The diagnosis of mixed infection can, of course, only be made when, in addition to crescents or to small or medium-sized tropical rings, full-grown or half-grown tertian or quartan parasites can be found.

I will now briefly describe the malarial parasites as they are observed in fresh preparations. I have purposely given first the description of stained preparations, which are of much greater importance to the practising physician than fresh preparations. *The diagnosis of malaria can, in fact, be made with certainty only through the examination of stained preparations*, the examination of fresh blood entailing far more liability to error. Already in 1897 I pointed out that the examination of stained preparations was greatly to be preferred, and recommended it especially to beginners.

The description of the malarial parasites as seen in fresh preparations, is, however, necessary for this reason, that the origin of one form —the flagellated body—can be observed in fresh preparations only.

The examination of fresh blood is performed in the following manner: a minute droplet of blood is added to a large drop of

* Tertian and quartan parasites are, however, much more rarely seen together.

normal salt solution (6 parts per 1,000), the cover-glass is ringed with vaseline, and the preparation is observed at the temperature of the room.

The Tertian Parasites

may be observed shortly after the onset of the fever, as minute bright organisms, almost invariably perfectly round, and without any recognisable structure. Their diameter is about one-fifth of that of an erythrocyte. There is nothing characteristic about their appearance, and they may readily be mistaken for small vacuoles. 'This youthful stage alike of the quartan and of the tertian parasites is difficult to observe, the substance of the parasites being but slightly differentiated from that of the erythrocytes' (Ziemann). It is not until twelve hours later, when the first minute motile granules of pigment make their appearance, and amœboid movements can be made out, that these parasites can be diagnosed with certainty. The amœboid movements, which can in some cases be made out even in the most minute forms, are, however, not alone sufficient to prove that the minute bright specks are parasitic in nature. For the small vacuoles occasionally manifest a perceptible movement, pulsatile in character.* The diagnosis can be definitely established only by the recognition of one or more granules of pigment. This becomes easier when the parasites are twenty-four hours old, for while the amœboid movement has now become less active, the pigment is more abundant, and often moves in a lively manner. The parasite, a light grey body, occupies about half of the infected erythrocyte, which is already considerably enlarged and decolourized. A strongly refracting speck in the parasite is by many authors regarded as consisting of chromatin. Shortly before the new paroxysm of fever, the enlarged erythrocyte is represented only by a pale inconspicuous ring surrounding the parasite, while the pigment is either distributed in irregular radiating lines or else aggregated into a mass. In these forms the development of the young parasites may be observed, appearing in the grey protoplasm of the mother-cell as isolated, minute, oval, brightly shining specks. They gradually increase in number until the mother-cell is completely filled with them. The enveloping erythrocyte then ruptures, the young parasites pass into the blood plasma, and the remaining pigment is taken up by the leucocytes.

* Translator's Note: This pulsatile movement, seen best in large vacuoles, is very characteristic, and can hardly be mistaken for the amœboid movements of a parasite, when once the observer has become familiar with it. We have called it the 'hippoid' movement, from the manner in which it recalls the rhythmical contraction and dilatation of the iris known as hippus.

The Quartan Parasites

develop in a similar manner, but in their case the process occupies seventy-two hours, the infected erythrocytes are neither enlarged nor decolourized, the amœboid movements of the parasites are hardly perceptible, but the parasites themselves are more readily visible than the tertian parasites; pigment also is more freely formed by the quartan parasites, and can already be perceived twelve hours after the onset of the fever.

In addition to the forms already described, both in the case of the quartan and in the case of the tertian parasites, large spherical forms are also seen, which in fresh preparations closely resemble mature parasites in appearance; these are either free in the blood or still enveloped by the remains of the erythrocytes; and they show no tendency to division. The pigment, which is scattered throughout the substance of the parasite, shows a very striking, active movement, known as 'swarming.' These forms are the so-called spheres, being identical with those already described (p. 10), as differentiated by staining reactions into two varieties according to the quantity of chromatin they contain and the manner in which their protoplasm takes up the stain.

After a time, about twenty minutes after the slide is first prepared, some of the free spheres become affected with spasmodic movements, are jerked to and fro several times, and then protrude from their margin fine filaments, four to six in number, and twice to three times the diameter of the sphere in length. These filaments, known as flagellæ, lash vigorously in a serpentine manner, pulling the body of the parasite actively in various directions, and finally tear themselves loose, and swim away with great rapidity. Their subsequent destiny will be discussed later.

The flagellated forms are met with in all three species of parasites.

The Parasites of Tropical Fever

are, in fresh preparations, much more difficult to recognise than the larger varieties of parasites; for the tropical parasites may be present in very small numbers only, so that their presence may be entirely overlooked. Moreover, the small and medium-sized tropical rings contain no pigment; and in the case of the large tropical rings isolated granules of pigment can be made out with difficulty only thirty to forty hours after the onset of the fever. It is only the

crescents, the spheres,* the flagellated bodies, and the fission-forms, that are easy to recognise, and are not liable to confusion with other forms. (The fission-forms, however, of the tropical parasites are met with only in blood taken from the internal organs.) The ring-forms, in fresh preparations, made during the height of the fever, are exceedingly minute, about one-sixth of the diameter of an erythrocyte; while, during the decline of the fever, they appear as small, bright, rather strongly refracting rings, with a diameter of one-third that of an erythrocyte, and usually placed near the periphery of the infected corpuscle (Ziemann). Their amœboid movement is not always obvious. The small rings may resemble ring-shaped fissures in the stroma of the erythrocyte; they may therefore readily be mistaken for small vacuoles. Hence it is impossible to establish a definite diagnosis by the examination of fresh preparations only, unless crescents, spheres, flagellated bodies, or fission-forms are observed. The three first-named forms, however, are seen only in fevers of long standing, whilst the last-named are usually to be found only in the blood of internal organs, such as the spleen, the brain, and the red marrow of bones. But we have not to deal only with cases of fever of long standing, nor can we make a routine practice of splenic puncture for diagnostic purposes.

From the preceding description it will be seen that I consider that there are three different species of parasite. These three species are clearly differentiated; and more especially are the tertian and quartan parasites (the larger species) notably different from the small tropical parasites. The difference between these two chief classes is strikingly obvious. Further, one particular form—that of the crescents—is found only in the tropical fevers caused by the small parasites; and if a crescent is seen in association with tertian or quartan parasites we have to do with a combination (*cf.* p. 13) of two different species of parasite (mixed infection). That the crescents are veritably forms developed only from the small species of parasites is established by the fact that in cases of tertian fever acquired in Germany in patients who have never been exposed to infection by tropical parasites, crescents are never met with.

Notwithstanding this, certain observers are still of opinion that there is but a single polymorphous species of malarial parasite, and this is the view of Laveran himself, the discoverer of the malarial parasites.

* As we have already shown, in the larger varieties of parasites crescents are not met with, and the spheres are formed directly in the erythrocytes without passing through the intermediate form of the crescent.

I may further state that the malarial parasites are invariably to be found in cases of malarial fever, and that they are never found in any other disease. They must therefore be regarded as the exciting cause of malarial fevers. I make this apparently superfluous statement for the reason that again and again, and down to the present time, certain misguided efforts have been made to deny the causal relation of the malarial parasites to the disease.

We have now completed the description of the life-history of the malarial parasites within the human body (endogenous cycle). In this description I have included an account of the formation of the flagellated bodies, because these bodies are met with during the observation of fresh preparations. The flagellated bodies, however, belong, not to the endogenous, but to the exogenous cycle (development in the mosquito). This latter cycle of development—that is, the further development of the malarial parasites outside the human body in an independent host, the anopheles genera of the mosquito—has been established only within the most recent times.

B. Life-history of the Malarial Parasites in the Mosquito.

The idea that mosquitoes can convey malaria is not a new one. In early times it was very variously asserted, but could neither be proved nor disproved with certainty. It was only in 1897 that Ross, following up a suggestion of Manson's, succeeded in observing the further development of human malarial parasites in two species of anopheles mosquitoes. Finally, in 1898, he was successful, not only in tracing in the common mosquito (*Culex pipiens*, Van der Wulp) the complete life-history of proteosoma (*Hæmamœba relicta*), a blood-parasite found in birds, belonging to the true malarial parasites, but he was further able to inoculate healthy birds with these parasites by means of the bites of infected mosquitoes. These observations were confirmed by R. Koch. Meanwhile, to the fundamental studies of Ross have been superadded experiments on the transmission of human malarial parasites by means of mosquitoes of the genus anopheles. Grassi, Bignami, and Bastianelli in Italy, Ross himself in further researches in Sierra Leone, and Ziemann in the Cameroons, succeeded in transmitting human malarial parasites to anopheles. Simultaneously, R. Koch was led by his researches to the conclusion that the transmission of human malarial parasites can be effected solely by the intermediation of anopheles.

It is, therefore, urgently necessary to consider briefly the genera of mosquitoes concerned in the transmission of these parasites, and

to describe their development, their morphological characteristics, and their mode of life, as a preliminary to the description of the life-history of the malarial parasites in these hosts.

According to the observations hitherto made, the transmission of the malarial parasites is effected solely by culicidæ belonging to the order of diptera, and of these two genera only appear to be effective agents—namely, culex and anopheles. The most common kinds of mosquito, with the appearance of which everyone is familiar, belong to the first-named genus. I shall, however, not merely describe the differences between the genus culex and the genus anopheles, but shall further describe minutely those species that are concerned in the transmission of malaria.

The differences between the two genera, culex and anopheles, begin already *ab ovo*. Whilst the eggs of the former are laid in variously-formed aggregations, those of *Culex pipiens* (Van der Wulp) having on superficial inspection a certain resemblance to mouse-dung, the eggs of anopheles, lying side by side, spread out over the surface of the water to form a kind of pellicle. The larvæ that proceed from these eggs are also clearly distinguishable from the outset. While the culex larvæ hang vertically beneath the surface of the water, with the head uppermost, the anopheles larvæ swim with the body extended horizontally beneath the surface. This difference in position depends upon the different length of the breathing-tube in the two genera. In culex it protrudes from the body at an angle of about 45°, and in order to bring the opening of the tube to the surface the larva must place itself in a perpendicular position. In anopheles larvæ the breathing-tube is so short that in order to breathe the larvæ must lie in a horizontal position.

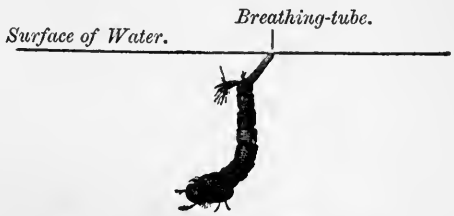


FIG. 1. — LARVA OF CULEX, MAGNIFIED 4 DIAMETERS, AS IT LIES BENEATH THE SURFACE OF THE WATER.

The larvæ inhabit small pools of stagnant water containing a luxuriant growth of algæ. Whilst the culex larvæ are less fastidious, and can be found in any accumulation of water, even if very small—such, for instance, as old tins in which a little rain-water is lying—anopheles larvæ are rarely found except in small ponds protected from direct rainfall and from wind, and containing large quantities of algæ. In rapidly-running water the larvæ of mosquitoes are not to be found.

The larvæ undergo transformation into pupæ (Fig. 3), which also live in water, and from these the winged insects proceed.

When we proceed to examine the full-grown specimens of the genus culex we find the males (♂) and the females (♀) clearly distinguished one from another by external characteristics. On the

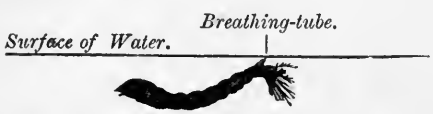


FIG. 2.—ANOPHELES LARVA, MAGNIFIED 4 DIAMETERS, LYING HORIZONTALLY BENEATH THE SURFACE OF THE WATER.

FIG. 3.—PUPA OF CULEX PIPIENS (VAN DER WULP), MAGNIFIED 4 DIAMETERS, LYING BENEATH THE SURFACE OF THE WATER.

small head of the female the long proboscis (*f*) is an obvious feature. That, however, which on examination with the unaided eye appears to us to be the proboscis is not the true proboscis, but merely a sheath, in the interior of which lies the proboscis itself. This sheath has above two lips, which can be separated from one another, the

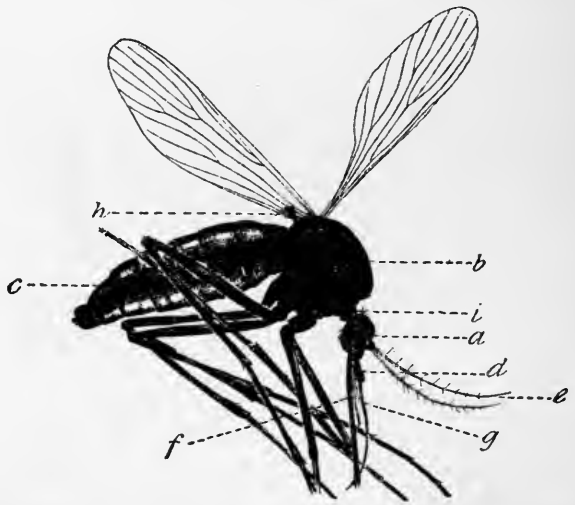


FIG. 4.—CULEX PIPIENS (VAN DER WULP), MAGNIFIED 8 DIAMETERS.

a, Head ; *b*, thorax ; *c*, abdomen ; *d*, palps ; *e*, antennæ ; *f*, proboscis sheath ; *g*, the true proboscis ; *h*, halteres ; *i*, neck.

true proboscis protruding from between them in the form of a hollow needle (*g*). Surrounding this are from four to six bristles, the function of which is probably to enlarge the wound made by the proboscis. Springing from the head, right and left of the base of the proboscis, are two very short palps (*d*). They are covered with bristly

hairs. Externally to the palpæ on either side are the antennæ. They have fifteen segments, having a ring of short hairs at the root of each segment, and their length is about three-fourths of that of the proboscis; while the palpæ have a length of about one-eighth only of that of the proboscis. On either side of the head are the two large compound eyes. Behind the head is the short, thin neck (*i*), springing

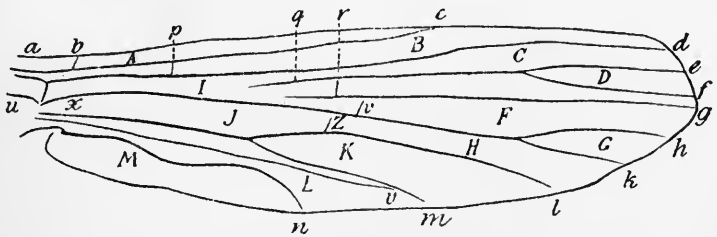


Fig. 5.—After Giles.

from the rectangular thorax, while posteriorly the thorax passes into the segmented abdomen (*c*).

The wings are attached to the middle of the thorax on either side. The indigenous species of culex, with two exceptions (*Culex annulatus* and *Culex glaphyropterus*), have unspotted wings. The wings are marked by an extensive network of vessels. The arrangement of these vessels varies to some extent, and may be employed in the determination of the different species. Each wing has two main

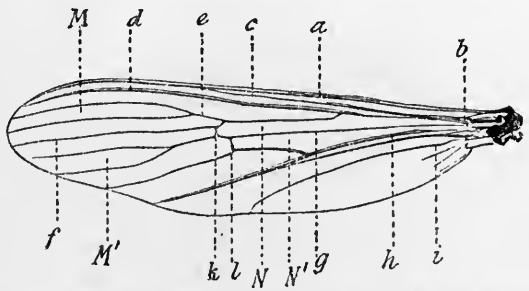


Fig. 6.—Wing of Culex pipiens (Van der Wulp), magnified 20 Diameters.

vessels, an upper and a lower, which branch but do not anastomose. The network thus formed on the wing is identical in the two sexes; but this is not the case as regards the spots on the wings, which in the males are less conspicuous than in the females. In order to determine accurately the position of these spots, it is necessary to be acquainted with the distribution of the vascular network. To an illustration of a wing I therefore append a brief nomenclature.

A. Nomenclature according to Loew (See Fig. 5.)	B. Nomenclature according to Van der Wulp. (See Fig. 6.)*
ag costa	vena costalis (*a*)
b vena transversa numeralis	,, transversa basalis (*b*)
c ,, auxiliaris	,, mediastinalis (*c*)
p ,, subcostalis transversa	
d ,, longitudinalis prima	,, subcostalis (*d*)
sef ,, ,, secunda	,, radialis (*e*)
tg ,, ,, tertia	,, cubitalis (*f*)
xlik ,, ,, quarta	,, discoidalis (*g*)
xlm ,, ,, quinta	,, posticalis (*h*)
n ,, ,, sexta	,, analis, axillaris (*i*)
q ,, marginalis transversa	
r ,, supernum, ,,	,, transversa media (*k*)
v ,, media ,,	
z ,, posterior ,,	,, ,, discoidalis (*l*)
	upper fork cell *M*
	lower ,, ,, M^1
	upper basal ,, *N*
	lower ,, ,, N^1

In the remarkably long and slender legs (Fig. 7) we distinguish: the thigh (femur), articulated to the hip by the so-called femoral ring; the shank (tibia); and the foot (tarsus), which is always made up of five segments. The proximal of these segments is almost as long as the four distal ones together, and is called the metatarsus. The terminal segment of the foot has two claws at its extremity.

The male culex (see Plate II., 3 and 4), differs from the female in the shape and length of the palps and in the hairiness of the antennæ. The palps are one and a half times as long as the proboscis, and their two terminal segments are bent upwards like the tusks of a boar. They are thick, and covered with moderately long hairs. The antennæ have a circle of long hairs around the base of each segment. The males are smaller than the females, and more delicately made.

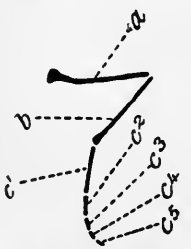


Fig. 7.—Leg of Culex annulatus, ♀, magnified 8 Diameters.

a, Thigh (femur); *b*, shank (tibia); c^1–c^5, the five segments of the tarsus.

The genus anopheles (see Plate II., 6 and 7) is at once differentiated from the genus culex by the fact that in both sexes the palps are of the same length, and are also of the same length as the proboscis. In the female, the two palps are usually placed so close to the proboscis that this either appears to be much

* The wing is figured without scales, because the individual vessels are thus shown more clearly. The transverse vessels especially are liable to be completely hidden by the overlying scales.

thickened, or else, if one of the palps stands out a little in a different direction, the proboscis looks as if it bifurcated (*cf.* Fig. 8). In the anopheles, the difference between male and female is indicated by the hairy character of the antennæ.

We may thus summarize the difference between the two genera:

In culex, palps in the female very short; palps in the male one and half times as long as the proboscis.

In anopheles, palps of identical length in both sexes, and of the same length as the proboscis.

In the males of both genera the antennæ are hairy. It is further to be noted that most species of anopheles hitherto described have spotted wings (*Anopheles bifurcatus* is an exception), whilst the wings of culex are unspotted, with the exception of the two genera already mentioned.

On superficial examination, *Culex annulatus* and *Anopheles maculipennis* (Meigen) cannot readily be distinguished from one another. They are almost identical in size, and each wing has four spots similarly distributed. In *Anopheles maculipennis*, however, the female as well as the male has long palps, whereas in the female of *Culex annulatus* these organs are very short. Moreover, in *Culex annulatus* there are yellow and black ringed markings on the legs, by means of which the males of this species may be distinguished from those of *Anopheles maculipennis*.

FIG. 8.—HEAD OF ANOPHELES MACULIPENNIS, ♀ (MEIGEN), MAGNIFIED 8 DIAMETERS.

The antennæ (*a*) are not hairy; proboscis and palps in such close apposition that the proboscis appears bifurcated; proboscis and palps of the same length.

There is another characteristic that enables us to distinguish at once between culex and anopheles. When at rest on a wall the culex stands with its body parallel, or nearly so, with the surface (see Fig. 9); whilst under similar circumstances the body of the anopheles forms an angle of 145° with the surface (Fig. 9), and the posterior extremities are stretched out into the air as if the insect wished to kick something.

As regards the habits of mosquitoes, the following points are of importance:

1. Only the females suck blood. The males live on a purely vegetable diet.

2. Mosquitoes are nocturnal in their habits. They begin to swarm at sunset, and fly about till dawn.

3. In the daytime they sit hidden in the grass, or on the under

surface of leaves, or on the bark of trees. They avoid wind, and seek sheltered quarters.

4. The fertilized females of our indigenous mosquitoes hibernate in human dwellings, in cellars, or in the stalls of cattle. The males die off in the cold weather. In Central Germany the females in mild winters leave their winter quarters as early as February, for the purpose of sucking blood; for it is only when the mothers have sucked blood that the fertilized eggs undergo any further development. By the end of March all the females have left their winter quarters. The young generation of mosquitoes makes its first appearance in April or May, according to the nature of the season. It has not yet been determined how the mosquitoes in the tropics pass through the dry season.*

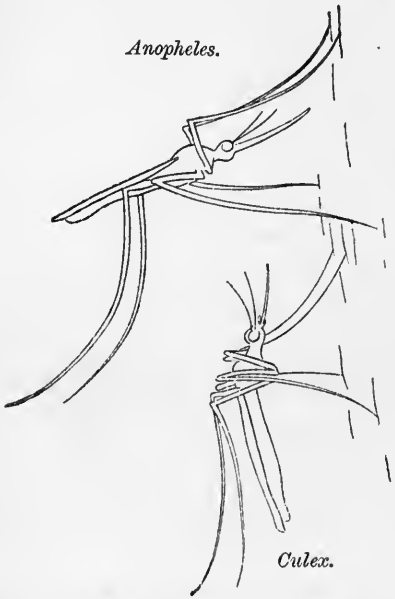


FIG. 9. AFTER A SKETCH IN THE "BRITISH MEDICAL JOURNAL," 1899.

The position of the anopheles is exaggerated.

5. The females of the genus anopheles in the summer also inhabit by preference human dwelling-places, in which they have the opportunity of sucking blood.

6. After sucking blood, the female anopheles usually remain for some time in the room in which they have made their meal. But since they usually enter houses at night, those whose blood they suck are most commonly sleeping; hence they are found chiefly in bedrooms, in dark corners, and especially sitting in old spiders' webs.

7. It is a mere fable that mosquitoes (including anopheles) suck blood once only, then lay their eggs, and die. Even in mosquitoes in captivity, blood-sucking and laying of eggs may be observed again and again.

It is, however, only in *Culex pipiens* (Van der Wulp) that the habits can be observed without difficulty in captivity.† The insects are best

* According to Christophers and Stephens it is probable that they pass through the dry season in the dirty huts of the natives.

† Quite recently, Van der Scheer kept a specimen of *Anopheles maculipennis* (Meigen) thirty days in captivity, allowing it to suck the blood of a rabbit.

collected in the autumn, when they find their way into cellars in order to hibernate. They must not be too rapidly transferred to a warmer room, as if this is done they will die. Even when they are gradually accustomed to a warmer environment they will not begin to suck blood until, on an average, fourteen days have elapsed, for they do not become hungry until their winter fat has been consumed. During this interval the gauze cages in which they are confined must be sprinkled with water every day.

Those mosquitoes that have sucked blood are readily recognised by the thickness of their body, through the wall of which the consumed blood appears of a bluish-black colour. They are then removed from the gauze cages in test-tubes, and placed in glass vessels with gauze tied over the mouth. In the bottom of these vessels is placed a cup of water, in which the eggs are to be laid. Each glass should be inscribed with the date on which the mosquito was fed, and the name of the animal from which the blood was obtained. Usually on the third day after the insect has sucked blood the first eggs are deposited. The culex larvæ hatched from these eggs will develop into mature insects in any water containing decaying leaves, moss, and water plants, the time occupied in the process varying from two to four weeks, according to the temperature and the nutritive qualities of the water. The males are the first to appear, but soon die unless there are growing plants in the cage. The females appear somewhat later.

The mosquitoes have also to be fed, and for this purpose sugar-water and slices of apple are most suitable. The gauze covering the glass must also be sprinkled with water daily. In this manner I have kept specimens of culex under observation for sixty days.

If the development of the vermiculæ of proteosoma is to be observed, the mosquitoes should be allowed to bite birds whose blood contains large numbers of parasites, as many as five in each microscopic field, since when the vermiculæ are as numerous as this they may be found without a prolonged search. If, however, sporozoites are required, the mosquitoes should suck blood containing a few parasites only, preferably not more than one or two in an entire microscopic preparation.*

* If sporozoites are required, infected sparrows are the best birds to use, because in these birds proteosoma-infection runs a chronic course, and the parasites are found sparingly. Sparrows in captivity, however, must be kept either alone or, better, in company with canaries; for if a number are placed in one cage they will either dash themselves continually against the bars of the cage, or else crawl into a heap and crush one another to death.

Since one mosquito can swallow as much as $\frac{3}{4}$ cubic millimetre of blood, it is not surprising that fifteen or more cysts may often be found in the stomach of a mosquito which has bitten a bird infected with proteosoma, notwithstanding the fact that in a microscopic preparation of the blood of the same bird we could perhaps find but a single specimen of the parasite. If, however, many cysts develop in the mosquito, the insect dies.

If subsequent to infection the infected mosquitoes are placed in the cages of healthy birds, from three to five days will often elapse before they bite again. Shortly after this they lay eggs for the second time, and even after this not more than 75 per cent. die in captivity.

After this brief account of the principal characters and habits of life of the mosquitoes with which we are concerned, we shall, in order to give some notion of the difficulty of determining the species of mosquitoes, proceed to give an exact description of the commoner kinds of mosquito, and shall then describe those species which may readily be mistaken for one another. Subsequently we shall describe the internal organs of the mosquito in which the malarial parasites complete their development.

The common mosquito, *Culex pipiens*, is described by Van der Wulp as follows:

From 4·5 to 5·75 millimetres in length, the antennal tuft of the male is dark or light brown in colour. The palps are brown, darker at the extremities in the male than in the female, with a thick but not shaggy covering of hairs. The two distal segments are of equal length, and are slender. The thorax is brown, covered with short reddish-yellow or rather lustrous golden hairs, and at times shows a trace of darker longitudinal striation. Posteriorly the hairs are longer, and a few of them are black. Laterally the thorax is pale, and covered with whitish hairs. The abdomen is dark brown, the margin of the segments being white or pale yellow. The claspers of the male are as long as the last segment, covered laterally with white hairs. The ventral surface of the abdomen is lighter, even pale yellow in colour. The legs are light reddish-yellow; the terminal extremities of the thighs, shanks, and tarsi usually brown; the shank of the hindermost extremity in the male covered with hairs on the external aspect. The halteres are pale yellow, the knobbed extremities of a darker colour. The wings have a vitreous appearance, with brown or brownish-grey hairs along the veins. *The fork of the vena radialis is very long, with a short stem. Its base is nearer the root of the wing than the base of the lower fork cell. The second (lower) root cell is notably shorter than the upper.*

Culex nemorosus is 6·25 millimetres in length; head brownish-yellow; eyes surrounded by a white border; antennæ brown, as is also the antennal tuft of the male; proboscis and palps black or brownish-black. In the male, the proximal extremity of each segment of the palps is of a lighter colour; the hairs of the distal segments are black and bushy. On the sides of the thorax are white tufts of

hair, on the back more or less distinct dark-brown longitudinal striæ. The yellowish hairs on the scutellum and the posterior portion of the back are long and fine. The abdomen is dark brown, with broad white margins on the segments. The claspers of the male are longer than the terminal segment of the abdomen. The legs are yellowish; the thighs and shanks are brown at their distal extremities; the tarsi brown throughout. *The knees have a silvery white speckled appearance.* The halteres are yellow; the wings yellowish, with dark-brown scales. *The fork of the vena radialis has a long stem, its base and the base of the lower fork cell lying almost side by side. The second (lower) root cell is shorter than the upper. The female closely resembles the female of* Anopheles bifurcatus, *but may be distinguished from it by the comparative shortness of the palps.*

Culex annulatus is from 6·75 to 9 millimetres in length, the antennæ and antennal hairs of the male brown, the joints of the male ringed white and brown; palps light brown with pale yellow or whitish rings. The four distal segments are almost equal in length; the hairs of the middle segments are dense and bushy, with brown and whitish spots. In the male, the short palps are blackish-brown with white extremities. The thorax is dark brown, covered with thick, short, yellowish hairs, and shows three short, indistinct longitudinal striæ. The sides of the thorax are somewhat lighter, with whitish hairy spots over the hips. Abdomen blackish-brown, with yellow, white-haired margins to the segments. On the sides of the abdomen the hairs are yellow. The claspers of the male are of the same length as the terminal segment of the abdomen. In the female, the second segment of the abdomen usually exhibits a white longitudinal stria. Legs brown, hips and proximal ends of the thighs yellow, distal extremities of the thighs and the shanks white, with, in addition, a white ring near the distal extremity of the thigh, and similar rings at the proximal extremity and in the middle of the first tarsal segment, and also at the proximal extremity of the three succeeding segments. On the internal aspect of the hindermost shank in the male are found thick, short hairs. Halteres brown, with a brownish-yellow stem. Wings vitreous. The hairs* along the veins are dark brown, and in some places so dense *that they appear spotted, especially at the root of the vena radialis, at the extremity of the two root cells, and on the base of the fork cell. The medial and posterior transverse veins lie side by side, so that the two root cells are of identical length. The bases of the two fork cells are also side by side.*†

This species is very commonly met with, even in autumn and winter, but occasionally only in houses.

The description of *Anopheles claviger* (=*Anopheles maculipennis*) and *Anopheles bifurcatus* is given according to Schiner. This distinguished dipterologist, in his 'Fauna Austriaca,' p. 623 *et seq.*, says: 'According to the observations of Fischer, the larvæ live in water. The habits of these mosquitoes resemble those of the culicidæ, *but I am not aware that the females suck blood.*' In other respects, however, Schiner's description agrees almost word for word with that of Van der Wulp.

Anopheles.—Individuals of this species appear rather large, but have a general resemblance to the common mosquito. The head is rounded; occiput strongly developed; proboscis bristle-shaped, broad, projecting horizontally, longer than the antennæ; *palps in both sexes as long as the proboscis*, consisting of four segments (as in most nemoceres, there is a small additional segment at the proximal extremity, which I have not taken into account); the two terminal segments together

* On the veins of the wings there are no hairs, but only scales, which form spots, by the manner in which in certain regions they are thickly set.

† The characters by means of which the various species are most readily differentiated have been indicated by the use of italic type.

are shorter than the antepenultimate, and in the male are covered with bushy hairs. The palps consist of fifteen segments, the proximal segment being thick and disc-shaped, the succeeding ones thinner, and so twisted as to give the organ a plumose appearance; the two terminal segments are elongated and covered with short hairs: in the female there is a short, bristly whorl of hairs. Eyes hollowed out on the internal aspect, simple eyes absent. Thorax strongly arched, rather long, narrowing anteriorly, with no transverse suture; scutellum narrow, posterior part of back rather strongly developed. Abdomen long and narrow, consisting of eight segments; genital organs small, and but slightly prominent. Legs long and thin, almost glabrous. Wings narrow, with very hairy veins and margins, the veins, as in corethra (that is to say, the second and fourth longitudinal * veins), forked in front, the fifth longitudinal vein sending off an upper branch in front of the posterior transverse vein, bending somewhat upwards to terminate quite close to the margin of the wing; the two basal cells (identical with the root cells) complete; the discoidal cell absent.

Anopheles maculipennis (*vel claviger*).—Legs light rusty yellow, tarsi dark, in certain positions appearing blackish-brown. Wings of a very pale yellow tint, the longitudinal veins having dense and dark hairs. *In three places the hairs are gathered into tufts—viz., in the fork of the second longitudinal vein, and at the origins of the second and third longitudinal veins—in very well-preserved specimens a dark spot of hair may sometimes be seen in the fork of the fourth longitudinal vein,* $3\frac{1}{4}$ *to* $3\frac{3}{4}$ *millimetres in diameter.*

Anopheles bifurcatus.—Closely resembles the species last described, but the wings have no spots. It is equally common with *A. maculipennis.*

In determining the species of mosquitoes we must first turn our attention to the head and its appendages. The antennæ are of especial importance in the differentiation of the two sexes. To discriminate between different species we consider the arrangements of the veins and spots on the wings, and in males the relative lengths of the claspers and the last segment of the abdomen. Finally, we examine the legs, to note the presence or absence of coloured rings.

We will now proceed to describe the internal organs of the mosquito, in so far as they are concerned in the development of the malarial organisms. In this connexion we have only to consider the stomach and the salivary glands.

The adjoining figure (Fig. 10) shows the abdominal viscera of a healthy female mosquito. The long tube in the right of the drawing is the œsophagus (*a*), which passes into the spindle-shaped stomach (*b*). The coiled intestine (*d*) proceeds directly from the stomach. Close to the posterior extremity of the stomach, five serpentine structures (*c*) open into the intestine. These are the so-called Malpighian tubes, which function as kidneys, and are named after the Italian anatomist Malpighi, who discovered them more than two hundred years ago. Not far from the posterior extremity of the intestine are the two ovaries (*e*). The viscera just described can easily be drawn out of

* *Cf.* Nomenclature on p. 20.

the body of a mosquito, while a preparation showing the salivary glands cannot be made except under the microscope.

In order to prepare the stomach and intestine of a mosquito for examination the only instruments needed are a couple of ordinary dissecting needles. With these the insect is placed on a glass slide, lying on its side in a large drop of normal salt solution, the wings and legs are pushed aside, the point of one of the needles is thrust into the thorax, whilst with the other the terminal segment of the abdomen is carefully detached by pressure, and with equal care completely separated from the rest of the body. It is advisable, for the performance of this operation, to have the needle with which the terminal segment of the abdomen is removed somewhat blunted, or turned up at the point, so that penetration of the abdominal cavity may be avoided. As the terminal segment of the abdomen is being drawn away, we notice at once that two white flakes, the ovaries, very small, but visible to the unassisted eye, remain attached to the segment; and that this latter is now connected with the anterior part of the body only by means of a fine white thread, the intestine, in which, in recently killed mosquitoes, peristaltic movements can readily be observed. By continued careful alternate traction and relaxation of the terminal segment of the abdomen we soon succeed in drawing out a coil of white threads—the Malpighian tubes, and great care must now be exercised, in further traction on the last segment of the abdomen, to avoid tearing the intestine across. If we feel that the tension is becoming too great, and are afraid that a rupture of the intestine is imminent, we must with the anterior needle separate the thorax from the abdomen, and with the same instrument we must then divide the œsophagus, for the intestine is much more easily torn across than the œsophagus. After the thorax has been separated, we must hold the first segment of the abdomen at its edge with one needle, and with the other we must withdraw the intestine attached to the terminal segment, if this separation has not already been

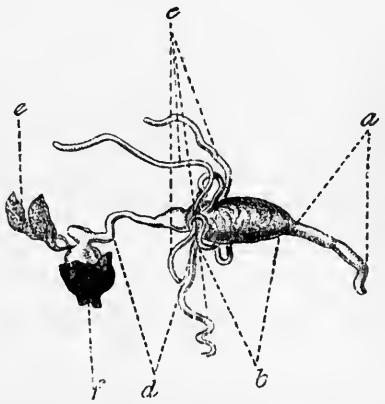


FIG. 10.—VISCERA OF A MOSQUITO, MAGNIFIED 15 DIAMETERS.

a, Part of œsophagus; *b*, stomach; *c*, Malpighian tubes; *d*, intestine; *e*, the two ovaries; *f*, terminal segment of the abdomen.

effected simultaneously with the detachment of the thorax. If the preparation is successful, we see under a low power of the microscope (objective 3, Leitz) that the stomach lies beneath the Malpighian tubes, and is covered with a network of tracheæ. Attached to the stomach is a portion of œsophagus, longer or shorter according to the place at which the thorax was divided.

The withdrawal of the viscera must be effected upon a dark background, because they appear white by reflected light.

If, however, we wish to demonstrate the very beginning of cyst-formation in the stomach of mosquitoes that have sucked blood containing malarial parasites, we must make a preparation of the stomach filled with blood, for even at a high temperature the blood in the stomach has not been fully digested and absorbed in forty-eight hours, after which period the first beginnings of cyst-formation in the external wall of the stomach are already to be made out. The digestive process in culex is even slower than this, for in specimens of this genus we often find an undigested residue of blood in the stomach as late as four days after its ingestion. In anopheles, however, digestion is usually completed in from forty-eight to sixty hours.

In the stomach distended with blood, which then has the appearance of a blackish-red ovoid body, the small cysts cannot be seen until the organ has been emptied. This is done by washing the preparation in a considerable quantity of normal saline solution. A glass slide is then plunged slantwise in the fluid beneath it, and the distended stomach is allowed to sink gently on to the surface of the slide, which is then lifted from the basin. The stomach then bursts, some of the blood running out. If the stomach does not burst spontaneously, it must be perforated with a needle. Enough salt solution is now added to enable a cover-glass to float on the surface of the preparation, and move to and fro easily without tearing the latter. Sufficient saline solution being repeatedly added, the cover-glass is gently raised and lowered until the stomach is quite emptied of blood.

The small cysts can then be recognised by the use of a $\frac{1}{12}$ inch immersion lens. If too little salt solution is used, the movements of the cover-glass either roll up the stomach into the form of a sausage or tear it in pieces. The examination then becomes much more difficult, because the spreading out of the rolled-up stomach is a very laborious process, in the course of which the little cysts are liable to be rubbed off. Moreover, pieces torn off from the stomach are apt to be turned right over, and the cysts, which are found on the outer wall of the stomach, cannot then be seen.

Minor difficulties also arise in the examination of the viscera of mosquitoes which contain developing ova, for in these insects the ovaries may be enlarged to from eight to ten times their original size. In such specimens, when the terminal segment of the abdomen has been separated and the intestine has been drawn out, a thick yellowish-white mass becomes impacted in the penultimate segment. This consists of the enlarged ovaries. These cannot be withdrawn in the simple manner described above, but the needle must be detached from the terminal segment, and must be used to stroke the thorax and abdomen from before backwards carefully with gentle pressure. In this manner the enlarged ovaries can be expressed, and the further withdrawal of the viscera can then be readily accomplished.

It may be added that the attempt to remove the viscera of a mosquito that has been dead more than twenty-four hours almost invariably results in failure. Successful preparations can be made only from recently-killed mosquitoes, a drop of ether being sufficient to kill the insects.

Fresh preparations of the viscera of mosquitoes may be preserved by simply surrounding the preparation in normal saline solution with a thick layer of glycerine. The glycerine gradually mixes with the saline solution. Such preparations will keep well for a year. It must be remarked, however, that the small pigmented cysts, which do not as yet contain sporozoites do not remain clear, but assume a granular appearance.

The preparation of the two salivary glands is a far more difficult matter than the simple withdrawal of the other viscera.

After the preparation of the abdominal viscera has been completed, the whole dorsal portion of the thorax, with the wings attached, is removed by a single incision running backwards in a line with the posterior border of the neck. This incision is made with a small curved scalpel, or, better still, with Frosch's knife, which has a knee-shaped bend. The remainder of the thorax is then transfixed with one dissecting-needle, while with another the head is seized and pulled backwards until it is detached from the thorax (see Fig. 11). In effecting this the mosquito must be placed on its side. The head and neck must be removed in one piece. When this preparation of the head and neck is examined under a low power, we see at the lower margin of the neck the ends of a number of tubes which have a finely granular appearance. These belong to the salivary glands. In an especially successful preparation some of the tubes of the salivary glands may already be seen to be almost completely unrolled. The

head is then cut across transversely, so that only that portion adjacent to the neck remains, and the salivary glands must now be dissected out under the microscope. One of the needles is thrust into the neck close to the head, and with the other needle we try to teaze out the salivary glands from the surrounding tissues. Hitherto we have been working in a considerable quantity of normal saline solution, in order to float out the tubes of the salivary glands (which otherwise tend to remain hidden in the surrounding tissues), and to render them more readily visible; but the completion of the preparation must now be effected in as small a quantity of fluid as possible. Indeed, the specimen must merely be well moistened, for otherwise the small objects we have to demonstrate will either cling to the dissecting-needle and be lost, or else will altogether elude observation.

FIG. 11.

The incision is indicated by a dotted line; the arrow shows the direction in which the detachment of the head must be effected.*

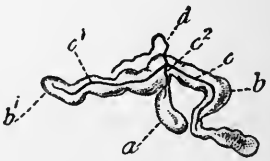


FIG. 12. — SALIVARY GLAND OF ANOPHELES MACULIPENNIS, ♀, MAGNIFIED 25 DIAMETERS. (PARTLY DIAGRAMMATIC.)

a, Medial lobe; *b*, *b*¹, lateral lobes; *c*, *c*¹, *c*², excretory ducts of the several lobes; *d*, common excretory duct (of the single gland).

In a really successful preparation—and such success is rare—the two salivary glands are teazed out together, remaining attached to one another by their common excretory duct. Commonly, however, the excretory duct is torn across, and the salivary glands are separately extracted.

A complete salivary gland consists of two large and long lateral tubes (*vide* Plate I., 50), surrounded by distinctly lobulated gland-tissue and a shorter medial lobe, the tissue of which has a granular appearance. Each of the three divisions has a separate excretory duct, the three ducts uniting to form a single duct, and this again joining with the corresponding duct of the other gland to form a

* This operation must first be practised on specimens of culex, which are easily obtained. It is for this reason that the illustration shows a culex instead of an anopheles.

common excretory duct. The principal duct thus formed opens into the anterior part of the œsophagus (*vide* Plate II., 15).

The preparation of the salivary glands, as long as the operation is conducted by the unassisted eye, must be performed over a white background, in order that the dark-coloured head and neck of the mosquito may be rendered distinctly visible. When the dissection has to be completed under the microscope, two ordinary dissecting-needles do not always suffice for our manipulations. It is as well to have two needles, the point of which for a length of half a millimetre is bent at an angle of 135° to form a small elongated hook (*vide* Fig. 13). With this implement very small structures can be held more easily, and with it there is not the same risk as with the ordinary straight dissecting-needle of crushing those parts of the salivary gland that have not yet been teazed out. Such little hooks, however, speedily become blunt, and must be frequently sharpened, for if they are not exceedingly sharp they do not fulfil their object, and crush the tissues quite as much as ordinary needles. The point must not be bent to a right angle with the stem, for if this is done the needle cannot be used for dissection under the microscope. We also need for incising the thorax the little knife with a knee-shaped bend invented by Frosch (*vide* Fig. 14).

FIG. 13.—HOOKED NEEDLE FOR THE EXTRACTION OF THE SALIVARY GLANDS, NATURAL SIZE.

A dissecting microscope is not required, for a little practice will render it easy to work by means of the inverted image of the ordinary compound microscope, and when the lowest lens of the objective (No. 3 Leitz) is unscrewed and put aside, we have so moderate a magnification that dissection with needles can be carried on without difficulty. Supporting-stages, which can be attached to the stage of any microscope, form a very convenient rest for the hands when dissecting, the space on the ordinary stage being too small to work upon conveniently, even when the slide is placed length wise on the stage, and not across it, as in ordinary microscopic work.

Since, notwithstanding all possible care, it may happen in the preparation of the salivary glands that one only of the two is successfully teazed out, or that the medial lobe, in which the sporozoites are especially numerous, is destroyed, it is wise to employ a different method of preparing suspected glands when we wish to determine with certainty whether the glands are, or are not, infected, for I have repeatedly observed that sporozoites existed in considerable numbers in the medial lobe of one salivary gland, while the medial lobe of the other gland was free from them, and at the same time none were to be found in the lateral lobes of either gland. For this reason, when we wish to ascertain indubitably the presence or absence of infection in the salivary glands of a mosquito, we have need of a method which will always enable us to examine the medial lobes of both glands.

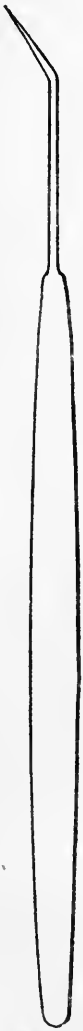


FIG. 14.—FROSCH'S KNIFE, WITH A KNEE-SHAPED BEND, NATURAL SIZE.

In such cases we must proceed as follows: The thorax and neck are separated from one another by an incision that leaves a small rim of the wall of the thorax attached to the neck. The neck is similarly severed from the head, leaving a small segment of the head attached to the neck (*vide* Fig. 15). In this manner both salivary glands are retained in the excised neck. The neck is then torn open with two dissecting-needles in a drop of normal saline solution, and is covered with a cover-glass, which is pressed down two or three times rather smartly, and the preparation is then warmed for an instant over the flame. The sporozoites thereupon exude from the crushed glandular tissue, and may easily be found.

This method has the additional advantage of celerity, and it can be employed with success even when the mosquito under examination has been dead from twenty-four to thirty-six hours, and is dried up or has begun to putrefy; for the sporozoites remain living for a long time, even after it has ceased to be possible to dissect out the salivary glands from the decomposing tissues. The method described by Italian authors, of expressing the sporozoites by pressure upon the head, is untrustworthy, because in this manner those sporozoites only are expelled which are already

in the common excretory duct of the salivary glands or in the proboscis.

For the preservation of fresh specimens of infected salivary glands we employ the method described on p. 29. The sporozoites, however, after the addition of glycerine, lose their sharp contour, and become shrunken (*vide* Plate I., 49).

Having now concluded our brief survey of the development, characters, and life-history of the mosquitoes concerned in the dissemination of malaria, and having also given an account of the methods of research, let us proceed to consider the exogenous cycle of the malarial parasites—that is to say, the stages of further development which these organisms undergo within the body of the mosquito.

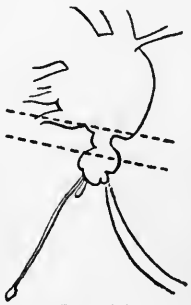


Fig. 15.

The investigations hitherto made render it probable that all the species of anopheles may serve in the transmission of the malarial parasites. But not every form of malarial parasite is capable of further development in the mosquito. The recognition of this fact is extremely important if we are to avoid being led to erroneous conclusions by means of fallacious experiments.

In the description of the malarial parasites as found in human blood, we have already drawn attention to the existence of certain forms characterized either by an abnormal development of chromatin (large parasitic forms) or by a peculiar shape (small parasitic form). In the case of the tertian and quartan parasites, we had to do with large, full-grown parasites, which either completely filled the containing erythrocyte, or else were quite free in the blood-plasma. These forms we named spheres or gametes. In fresh preparations they were recognised by the fact that their pigment was not, as in other full-grown parasites, aggregated in a mass in the centre or at the periphery of the parasite, but was distributed throughout the substance of the parasite in actively motile isolated little rods and granules. In preparations stained by Romanowsky's method, notwithstanding their great size, their chromatin displayed no signs of commencing division.

Among these forms, two kinds were further to be distinguished, one containing much chromatin, arranged in loose skeins, while their protoplasm was stained faintly blue; the other containing little chromatin, arranged in fine granules, with protoplasm stained a deep blue colour.

These forms are known as gametes (γαμέτης) because their function is the sexual reproduction of the malarial parasites without the human body, in the stomach of the mosquito. The first kind, that with much chromatin and faintly stained protoplasm, is the male parasite. For in this form, when we keep a series of preparations in a damp chamber, and stain these at suitable intervals by Romanowsky's method, we find that the chromatin is extruded in the form of motile flagella. On account of this formation of flagella they are called *microgametocytes*, the flagella themselves receiving the name of *microgametes*. These flagella represent spermatozoa, for they penetrate the gametes of the second kind—those with little chromatin and deeply-stained protoplasm—and fertilize them. Gametes of this second kind are therefore regarded as female individuals, and are known as *macrogametes* or ovoids.

This same process of sexual reproduction is in the parasites of tropical fever effected by means of the crescents. In these also we can distinguish two types, one containing much chromatin and with the protoplasm faintly stained; and the other containing little chromatin, but with deeply-stained protoplasm. The former are male parasites, which develop into spheres and extrude flagella (spermatozoa), and these presumably penetrate the crescents of the other type, which develop into spheres without extruding flagella.

The life-history of the human malarial parasites within the body of anopheles mosquitoes has not yet been observed in all its stages. But inasmuch as the first stage of the exogenous development may be directly observed under the microscope in another true malarial parasite (halteridium), and the further course of this development has not only been fully studied, up to and including the formation of sporozoites, in a third true malarial parasite (proteosoma), but has been found to correspond exactly with the development of the human malarial parasites, we shall describe the exogenous development of the human malarial parasites according to the observations made on halteridium and proteosoma.

The sexual reproduction of malarial parasites was first observed by MacCallum in 1897 in halteridium—a parasite met with in the blood of pigeons and nesting birds generally. These observations have been confirmed by R. Koch. The process of sexual reproduction is carried out in the following manner:

If to a small drop of normal saline solution (0·6 per cent. sodium chloride solution), containing 10 per cent. of the serum of pigeon's blood, we add as much halteridium-containing blood as can be taken

up on the point of a platinum wire, we observe that the halteridia soon become rounded, leave the corpuscles, and some of them assume the flagellated form. These numerous flagella, becoming free and swimming here and there about the preparation, then penetrate other parasites which have also left their enveloping corpuscles and become rounded, but without the formation of flagella, the penetrating flagella disappearing completely in their interior. After three-quarters of an hour or so we observe in these fertilized parasites, known as *zygotes*, the formation of a small conical projection, which gives the whole body a resemblance to a sprouting seed (R. Koch). This projection, continually growing larger, becomes bent like a horn, so that the originally round parasite assumes a vermicular form (*vide* Plate I., Nos. 39 to 42).

The further development of this halteridium vermicule has not yet been observed. But in another blood-parasite of birds, the already mentioned proteosoma, the growth of the vermicule into a cyst in the stomach of its host (*Culex pipiens*, Van der Wulp) can readily be observed.

If a *Culex pipiens* has ingested blood containing proteosoma, and has been kept at a temperature not less than 24° C., there will already at the end of twelve hours be fully developed vermicular proteosomata in the stomach of the insect. These vermicules pierce the wall of the stomach, and may be seen forty-eight hours later on the external wall of the organ as vitreous, round, or ovoid pigmented bodies, with a sharply-defined outline, and of about half the diameter of an erythrocyte (*zygotes* or *cysts*, also known as Ross's corpuscles). These globular bodies increase rather rapidly in size, so that at the end of five days their diameter is about six times as great as at first, and new little spheres have formed in their interior; these are named daughter-cysts by R. Koch, and meres by Ross. The contents of these daughter-cysts become changed on the sixth and seventh day into sporozoites, the daughter-cyst being then called by Ross a blastophore. The whole cyst has now a finely striated appearance. The cysts burst and the sporozoites are set free in the cœlom of the mosquito, being found on the eighth day in the salivary glands, and especially in the medial lobes of these. The development only proceeds in the manner here described when the temperature is maintained above 24° C.* The sporozoites expressed from the cysts in the stomach or from the salivary glands are actively motile.

* Professor Frosch found that in parasites of German sparrows the development of proteosoma will proceed in the stomach of a mosquito at a temperature ranging from 16° to 24° C., but it is then much slower than above described.

In fresh preparations the sporozoites expressed from the salivary glands are always found isolated, while those obtained from ruptured cysts in the stomach wall are usually found in groups of six, eight, or more in number, fastened together at one end to form a kind of fan, or arranged radially like the spokes of a wheel. The length of the individual sporozoite is from $1\frac{1}{2}$ to 2 diameters of an erythrocyte, and the length is about eight times the breadth. The shape is usually rather that of a lancet than of a sickle. The sporozoite is bright grey, and has a finely granular appearance. There are sometimes two bright spots, sometimes there is only one, in the interior. It is actively motile, now assuming the shape of a capital omega, now that of a capital P, now that of a sickle or of a ring, which last, however, soon breaks open. The sporozoites have a swarming motion, and move actively round the erythrocytes, without, however, penetrating them.

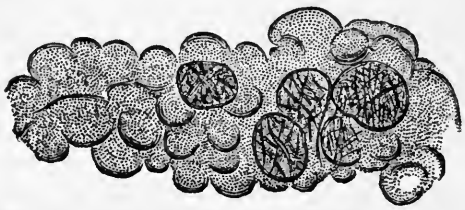


FIG. 16.—SPOROZOITES IN THE SALIVARY GLAND OF A MOSQUITO (DIAGRAMMATIC), MAGNIFIED 500 DIAMETERS.

In the medial lobe of the salivary glands they lie in a thick cluster, while in the lateral lobes they form a fine network. They are usually found in some only of the lobules of the gland, and either isolated, when they are readily recognised without staining, or else in clusters (*vide* Fig. 16). Care must be exercised not to mistake for sporozoites the small mouths of the numerous excretory ducts of the lobules opening into the common duct. Improbable as the confusion may seem, it is, in fact, easy to make in cursory observation. The error may, however, be easily avoided by following up the course of the ducts. The ducts of the lobules open almost invariably at right angles into the common duct, running in this direction for some little distance, while the sporozoites always lie irregularly over one another, crossing so as to form a net- or trellis-work. In the stomach, also, floating, rolled-up elastic fibres, or the minute endings of tracheæ, may be mistaken for motile sporozoites. If, however, we follow up the further course of these elastic fibres, the error becomes at once apparent.

Sporozoites stained by Romanowsky's method exhibit a large, oval chromatin granule in the middle, and dark-blue staining at the ends. The protoplasm around the middle is less deeply stained, as in the crescents of human malaria.

In addition to these forms, the nature of which is known, there are others the significance of which is not fully understood. These are Ross's spores (black spores). They are usually found in the stomach only,* in the neighbourhood of cysts about to form sporozoites These structures are brownish-yellow to blackish-brown in colour, bent in the form of an S, or comma-shaped, somewhat longer than the sporozoites, and at least twice as wide. Like the sporozoites, they lie together in cysts, and the cysts containing black spores have themselves a black appearance. Like the cysts containing sporozoites, the black spore cysts can readily be recognised with a low power (obj. 3, Leitz). The individual Ross's spores are not always well developed. They often assume the form of plump brown little rods, which are only one-half the size of the fully-developed black spores. Black spores and normal sporozoites are occasionally found together in the same cyst. For this reason Ross assumed that the former are developed from the latter. I have been able to prove that this view is correct by finding cysts containing yellow and brown sporozoites together with transitional forms between brown sporozoites and black spores. Whether these black spores are or are not degenerative products has, however, not yet been ascertained, for in hanging-drop preparations kept at the temperature of the room they will remain unaltered for as long as fifteen months, while, when incubated, they become ovoid in form in a fortnight. Inoculation experiments on the larvæ of mosquitoes have hitherto given no result.

We have now described the course of development of the blood-parasites of birds, which are numbered among the true malarial parasites. It is assumed that the course of development of the human malarial parasites in the anopheles mosquito is altogether similar. Cysts have indeed been found in the stomach-wall of anopheles of various species which had sucked the blood of patients suffering from malaria, and these cysts were hardly distinguishable from proteosoma cysts. The development of the sporozoites, and their migration to the salivary glands, were also observed to occur precisely as in proteosoma.

The course of development of the human malarial parasites within the human body is thus fully known, whilst the extra-human phases have been observed with some approach to completeness. Small gaps in our knowledge of the life-history are filled in by our observations on corresponding stages in the closely-allied blood-parasites of birds,

* In two cases only I have seen them in the salivary glands.

for these observations may be thus applied without hesitation. It is, indeed, directly established that the human malarial parasites undergo further development in anopheles, and that the terminal forms of this cycle of development, the sporozoites, accumulate in the salivary glands. The excretory ducts of the salivary glands open by means of a common duct into the upper part of the œsophagus, and the sporozoites can thus find their way into the proboscis in the process of blood-sucking, and so be inoculated.

That as an actual fact malarial fever can be transmitted by the bites of infected female anopheles has been established by various experiments. I need refer only to the most recent, made by Sir Patrick Manson in London in the autumn of 1900. He received from Italy female anopheles which had sucked blood from a patient suffering from tertian fever. Manson's son, himself a medical man, was bitten by several of these insects. He subsequently fell sick with a tertian fever. He had never previously visited any malarial district, nor had he ever suffered from malarial fever.

In conclusion, I append the synopsis of the two cycles of development of the malarial parasites drawn up by R. Koch[4]:

1. Very young parasites in the form of a chromatin body, which is surrounded by a small quantity of plasma. Pigment not yet formed. They live in or upon erythrocytes.

2. Half-grown parasites. The plasma has grown considerably more in proportion than the chromatin. Pigment-formation has begun.

3. Full-grown parasites. Further increase of chromatin, plasma, and pigment.

4*a*. Division into a number of young parasites, with extrusion of pigment.

The young parasites begin the cycle anew (1-4).

Endogenous cycle.

4*b*. Separation of the parasite from the erythrocyte. Recognisable differentiation of the parasites into male and female individuals.

Here begins the exogenous cyle in the intermediate host.

5. Process of fertilization in the stomach of the intermediate host.

6. Metamorphosis of the fertilized female parasites into vermiculæ.

7. Migration of the vermiculæ through the stomach-wall of the intermediate host, and their transformation into spheres resembling coccidiæ.

8. Formation of sporozoites in the spheres.

9. Deposit of the ripe and free sporozoites in the poison-glands.

10. Inoculation of the young parasites into the terminal host by means of the bite of the intermediate host.*

II. EPIDEMIOLOGY.

It has always been noticed that malaria becomes epidemic at certain seasons of the year, and that in hot climates the disease is much more severe, and of much commoner occurrence, than in temperate regions. It was also an obvious and incontestable fact that malaria was a disease far more prevalent in the open country than in cities; but it was soon observed, not only that the appearance of malarial fever was associated with the recurrence of certain seasons, but also that different times of day had a very varying influence on the liability to contract the disease.

Further observations made by numerous ships' surgeons from the middle of the eighteenth century onwards also established that those members only of a ship's complement who had been on shore sickened of malarial fever, whilst those who had remained on board, as long as the ship was not lying too close to the shore, were not attacked by the disease.

Since it was also known that on flat, marshy stretches of coast near the mouths of great rivers (I need only mention Senegambia, Lagos, or the mouth of the Congo) malarial fevers assumed a most deadly character, and that a soil which was alternately damp and dry was much more favourable to the development of the disease than a high, dry, rocky soil, it was natural that quite definite theories as to the origin, and quite definite measures for the prevention, of the disease should be formulated.

In this matter physicians were not all of the same opinion, but there were two theories only in the field.

According to one of these theories, to which at the present day many medical men still cling, the germs of malaria were to be found in the soil. This view was based, not only on the fact that among sailors those almost exclusively were attacked who had been ashore, whilst those who had not left the ship did not suffer from the disease, but also, and principally, on the circumstance that, after breaking up the soil, or after excavations on a large scale—as, for example, at the harbour-works of Wilhelmshaven—malarial fever immediately became

* See note, p. 3.

epidemic. It was said that the germs were contained in the soil, that through the breaking up of the soil they were set free in large numbers, became disseminated through the air, and were then inhaled.

On the other hand, this theory furnished an easy explanation of the fact that not only was malaria far more prevalent in the open country than in cities, but also that the frequency of the disease diminished *pari passu* with the increasing cultivation of the soil. It was supposed that cultivation led to the formation of a protective cover to the soil, a cover impenetrable by the germs of malaria. If we wished to avoid an epidemic of malarial fever, we must carefully avoid the removal of this protective cover from the soil. This theory also provided an explanation of the fact that malaria was more apt to occur on a damp soil, or, at least, on a soil alternately damp and dry, than on a dry soil. It was pointed out that moisture was essential to the development of micro-organisms. The occurrence of malarial fever in the hot season of the year was similarly accounted for, for a micro-organism needs for its development, not moisture only, but heat as well. The influence of heat on the appearance and the course of malarial fever was proved in a masterly manner by Wenzel, sometime Surgeon-General of the Navy, in 1871, in his treatise on 'Marsh Fever.' He found that at Wilhelmshaven, with a moderate summer temperature of 14° R. (64° F.), epidemics of malarial fever began, and that their commencement dated always twenty to twenty-five days later than the period of greatest heat. Of this time, he allotted ten to fourteen days to the period of incubation, and six to eleven days to the development of the micro-organisms which he suspected to exist in the soil. The periods given show the excellence of Wenzel's observations. They will occupy us again later.

The ground-air theory could not, however, offer any entirely satisfactory explanation of the fact that it was exposure to night-air which especially led to infection with malaria. It was suggested as a way out of the difficulty that the germs rose from the soil in the form of a nocturnal miasma.

Other authors, however, whose views were founded on a few isolated examples, maintained the theory that drinking-water was the medium of infection. Quite recently this view has been reasserted by Rogers, of the Indian Medical Service, because he has ascertained that in certain districts north of Calcutta the inhabitants of those districts the water-supply of which was good suffered in much smaller numbers from malarial fever than the inhabitants of districts supplied

with water from rivers and tanks only. Rogers, however, regarded enlargement of the spleen as evidence of infection with malaria; his researches were not supported by any examination of the blood.

In opposition to this view, it may be pointed out that the installation of a good water-supply has not, by itself, sufficed, in many malarial regions, to bring about a diminution in the prevalence of malaria. On the other hand, the following experiment has been made by Italian observers. Healthy people residing in non-malarial districts were given for several weeks water obtained from malarial regions, which they drank in a daily quantity of as much as three litres. These individuals remained healthy. Contrariwise, persons living in malarial districts were supplied with water from healthy districts, and none the less sickened with malaria. These experiments proved that the transmission of malarial fever was not effected by means of water.

In addition to these two theories, a third, less widely known, gradually developed. It was suggested by King in 1883 that mosquitoes were perhaps concerned in the dissemination of malarial fever. Since, however, at the time when this theory was formulated, our knowledge of the malarial parasites and also of the habits of mosquitoes was neither extensive nor intimate, the theory attracted very little attention. Manson, however, stimulated by the result of his researches into the nature of filariasis, followed this theory up; and Ross, who, as we intimated in the first chapter, inspired by Manson, occupied himself in research along these lines, succeeded eventually in proving that in certain mosquitoes (anopheles) the parasites of human malaria undergo a further development, and that other mosquitoes (*Culex pipiens*, Van der Wulp) are the veritable agents of transmission of the blood-parasites of birds. Finally, and quite recently, R. Koch was by his own researches led to the conclusion that human malaria is disseminated only by anopheles mosquitoes.

Does the hypothesis that anopheles mosquitoes disseminate human malaria afford a satisfactory explanation of the known facts of the epidemiology of malaria?

Let us briefly enumerate the epidemiological facts with which we are concerned, and show that they can all be explained in accordance with the hypothesis under consideration.

1. The fact that epidemics of malarial fever in the North German lowlands arise, according to Wenzel, from twenty to twenty-five days after the maximum heat of summer—provided that in this heat a mean temperature of 16° C. (61° F.) is attained—and further that in Italy,

according to Koch, epidemics begin three weeks after a temperature has been attained in the interior of houses of not less than 24° C. (75° F.); and, finally, that in the tropics epidemics begin about one month after the period of maximum rainfall—all these observations are readily explicable on the hypothesis that malaria is disseminated by anopheles. A high temperature, for a short period at least, is required to enable the development of the malarial organisms to proceed within the body of anopheles. If, in the comparatively low temperature of North Germany, we allow thirteen days for the development of the parasite in the mosquito, and twelve days for the period of incubation, the epidemic appearance of malaria twenty-five days after the maximum temperature of the previous month is satisfactorily explained. Wenzel, indeed, showed that after periods of exceptionally great heat malaria broke out in twenty days, an observation that harmonizes also with our theory that the warmer it is, the more rapidly proceeds the development of the malarial parasite in the body of the mosquito.*

This last observation is confirmed by the researches of R. Koch, who noted the sudden epidemic appearance of malaria three weeks after a maximum temperature of 27° C. (81° F.) had been attained. At this high temperature the development of the malarial parasites up to and including the migration of the sporozoites into the salivary glands is completed in nine days. The remainder of the interval before fever appears is occupied by the period of incubation.

2. Infection with malaria takes place for the most part at night. The anopheles is a nocturnal insect, flying about and sucking blood chiefly between sunset and sunrise, while in the daytime it hides in dark corners, and bites only when disturbed.

3. Malarial fevers are diseases of the open country, and not of cities. This is explained by the fact that anopheles find no suitable breeding-places in cities; hence they avoid the interior of cities, and are found in the environs only. But much more do they frequent the open country, where breeding-places are everywhere to be found.

4. The extension of buildings and increased cultivation lead to the extinction of malaria, because they destroy the breeding-places of the anopheles.

5. Upturnings of the soil are followed by the appearance of malarial fever. First of all, by the breaking up of the ground, the

* Quite recently this statement has been confirmed by some observations made in Holland by Van der Scheer, on the development of tertian parasites in *Anopheles maculipennis*.

anopheles are hunted out of their natural hiding-places, bite the workmen, and become diffused through the neighbourhood; and, secondly, fresh breeding-places are provided for them—little hollows in the ground in which puddles form, suitable for the deposition of ova.

6. In the northern civilized countries there has, during the last thirty years or so, been a most notable decline in the prevalence of malarial fever. This depends, in the first place, but in a quite minor degree, on the reasons given in Section 4; *chiefly, however, it is due to the more intelligent and more extensive administration of quinine.* All cases of malarial fever that occur being cured, the anopheles, which, despite the progress of cultivation, are still very numerous, have no opportunity of becoming infected.

7. The crews of ships, provided the ships do not approach the land too closely, suffer far less from malarial fevers than landsmen, so long as they remain on board their ship, or, if they go ashore, do so during the daytime only. The reason of this exemption is that anopheles do not fly far, and a few specimens only reach the ship. The farther off from a malarial coast a ship lies, the more healthy, under the circumstances described, will the crew be. According to Wenzel, cases of malaria continued to occur at Wilhelmshaven up to a distance of about five kilometres, or three English miles, from the harbour, the focus of virulent infection. This, then, would seem to indicate, under ordinary circumstances, the extreme limit of the flight of an anopheles. Local conditions must, of course, in all cases be taken most carefully in consideration, for local currents of air * may, under certain conditions, carry anopheles beyond this distance.

Certain other facts may be explained by the malaria-mosquito theory. In the tropics, the transition period from the dry season to the rainy, and the reverse, are regarded as times of exceptional danger. It was formerly said that the dangerous quality of these periods was due to the fact that they were especially favourable for the development of the malarial germs in the soil. Now, however, we can offer another explanation. The so-called small rains provide numerous breeding-places for the anopheles, whilst the heavy rains often overfill and wash out these puddles, thus destroying the brood. Light showers are especially dangerous when they occur just at sunset, when the anopheles have already begun to fly about in great numbers; for

* In speaking of air-currents we do not mean strong winds, for these latter do not lead to the dissemination of mosquitoes, because when they occur the mosquitoes hide themselves in grass and foliage.

these showers drive the insects into the houses, whereas heavy rains destroy them.

In opposition to the malaria-mosquito theory, the following considerations have been adduced:

1. There are certain fever districts of the very worst kind, as, for example, the Cameroons, in which no mosquitoes are found. But after physicians had learned how to find mosquitoes, it was shown that anopheles do, after all, exist in the Cameroons. Dr. Ziemann, Staff-Surgeon in the German Navy, was so kind as to send me a bottle containing mosquitoes found in the Cameroons. Amongst the fifty-two mosquitoes sent, there were three anopheles; whereas in a collection of mosquitoes from the Zanzibar coast, for which I am indebted to Dr. Bütow, Staff-Surgeon in the German Navy, of 246 specimens, one only was an anopheles.

Even more instructive in this connexion are the reports which Strachan has furnished from Lagos[5]. In this district, so notorious for the prevalence of malarial fever, he found anopheles almost exclusively, with merely a few isolated specimens of culex.

This objection, therefore, falls to the ground.

2. It has been objected to the malaria-mosquito theory that hitherto the transmission of malaria from one place to another has never been demonstrated to occur. I need merely remind my readers of the case of the islands of Mauritius and Réunion, in which malarial fever appeared for the first time in 1865 and 1869 respectively.* More careful investigation in the future is likely to bring a number of similar instances to light.

3. If by opponents of this theory it is suggested that the fact that in uninhabited wildernesses people sometimes are attacked by malarial fever does not harmonize with the malaria-mosquito theory, we must reply that hitherto in no single case has proof been afforded that the districts in which these cases of illness occurred were really uninhabited.

In this connexion we may examine the interesting observations of the English Expedition sent out by the Royal Society for the study of Malaria. In a patch of otherwise uninhabited jungle interspersed with marshes, in the neighbourhood of Freetown, Christophers and Stephens found some Europeans engaged in building a railway bridge over a river with marshy banks. All these Europeans were suffering from malaria, and they regarded their detention (when at work) on the

* Probably the infection was introduced by the Indian emigrant ship *Spunky*, on board of which were persons suffering from malaria.

banks of the river as the cause of their infection. In this region, however, Christophers and Stephens were able to find but a few isolated specimens of anopheles. In the dwelling-house of the Europeans, somewhat remote from the river bank, they found anopheles in greater, but still scanty, numbers. But in the adjacent huts of the native servants and workmen they found anopheles, not only in great abundance, but, further, they found them to be infected in a proportion of from 5 per cent. to 20 per cent.

It was, then, here, and not on the banks of the river, that the Europeans had contracted malaria.

4. A further objection may be urged, that anopheles bite also during the seasons of the year in which fever is not prevalent, and that their bites then do not promote an epidemic of malaria. That the anopheles bite at times in the year when fever does not prevail is certainly proved. But it is equally proved that in the cool, non-febrile seasons the further development of the malarial parasites does not take place in the anopheles. Koch reports that he has found sporozoites in the poison-glands of mosquitoes only during the hot season; whereas in the very numerous mosquitoes of the same species examined by him during the cooler seasons of the year nothing of the kind was ever observed.

We must, therefore, suppose that towards the end of the spring season the anopheles become freshly infected, from relapsed cases of malarial fever, and that, the temperature being now high enough for the purpose, the malarial parasites undergo the complete cycle of development in the body of the anopheles, which proceed by their bites to disseminate the disease.

R. Koch, therefore, urged that the link between the successive summer epidemics of malaria which is furnished by the relapsing cases should be eliminated by the methodical administration of quinine. Such a method would, however, be rational only if it had been proved that the circulation of the malarial parasites is from man to anopheles and anopheles to man only. That this is truly the case Koch was the first to establish, having examined the blood of a large number of animals, finding blood-parasites indeed, but human malarial parasites never.

As a result of these researches, he expressed the conviction that the life-cycle of the human malarial parasites is between man and anopheles only. Ross has come to the same conclusion. In the writings of the Italian observer Celli, who claims priority in this investigation, we find, however, not a single word referring to this principal argu-

ment with which the whole theory of the limitation of the cycle of the human malarial parasites to man and anopheles stands or falls.

I shall now add a few brief remarks on the period of incubation. On this subject much has been written of an apocryphal nature. In some reports the statement is made that persons newly arrived at a fever-stricken coast have been attacked the same day, within a few hours even, with intermittent fever. In these cases there has been confusion with heat-stroke or some other illness. For the period of incubation of malarial fever has been accurately determined by numerous inoculation experiments. On the average it is of twelve days' duration.* Mannaberg has collected a great number of these experiments. Gerhardt, in 1884, was the first to prove that malaria could be transmitted by inoculation of blood. In one of his cases the period of incubation was seven days, in the other eleven days. A further reason for rejecting the idea of a period of incubation in malarial fever of a few hours only is found in the fact that the cycle of development of the smaller malarial parasites occupies from twenty-four to forty-eight hours, and that of the larger parasites from forty-eight to seventy-two hours, and that the malarial parasites must multiply themselves for several generations, until they are sufficiently numerous to determine an attack of fever.

III. SYMPTOMATOLOGY.

Etiological considerations enable us to divide the malarial fevers into two great groups, which have likewise (in the case of newly-infected persons) distinctive clinical characters. The fevers evoked by the large species of parasites (tertian and quartan fevers) may be at once distinguished clinically from the tropical fevers evoked by the small species of parasites (tropical fever, summer-autumn fever, malignant tertian). But in chronic malaria the originally distinctive characters gradually disappear. I shall, therefore, first describe the acute malarial fevers, and discuss chronic malaria in a separate section.

A. Fevers caused by the Large Species of Parasites.

Preliminary Note. — In the following account by the term 'paroxysm' we denote the individual rises of temperature, lasting from six to twelve hours; while the term 'attack' denotes a series

* In rare cases twenty days.

of successive paroxysms of this character (*vide* Temperature-Charts I. and VI.).

In these fevers, a prodromal stage may be apparent, characterized by general malaise, lassitude, yawning fits, weight in the limbs, and headache. But prodromata may be entirely lacking. When the illness is fully developed, we have to do with intermittent fever in the proper sense of the term, consisting of relatively short, sharp paroxysms of fever, separated from one another by an afebrile period, and characterized successively by rigor, heat, and sweating.

Frequently persons till then in the best of health are suddenly seized with a severe rigor, the temperature running up like a rocket even to 41° C. (105·8° F.). The rigor is succeeded by the hot-stage, after which, with the appearance of profuse sweats, the temperature falls to the normal with as great rapidity as it had previously risen. It may descend below the normal, even to 35·5° C. (95·7° F.). The duration of the whole paroxysm is on an average from six to eight hours; in exceptional cases from twelve to fourteen hours.* The following physical signs are observed. During the cold stage the face is pale, pale also the skin of the rest of the body. During the hot stage the skin becomes red and somewhat turgid, and to the touch it is hot and dry. The frequency of the breathing is increased, the pulse-frequency rising to 90 or more per minute. The patient complains of headache, and even in these simple paroxysms of intermittent fever when the fever is at its height there may be moderate delirium. The lips and tongue are dry, the latter being more or less thickly coated; intense thirst is complained of, appetite is lost completely, and even small quantities of citric acid lemonade may cause vomiting. In the heart and vascular system, and also in the respiratory system, no morbid signs are to be observed beyond the moderate increase in the frequency of pulse and respiration. The urine contains albumen rarely, and casts never.

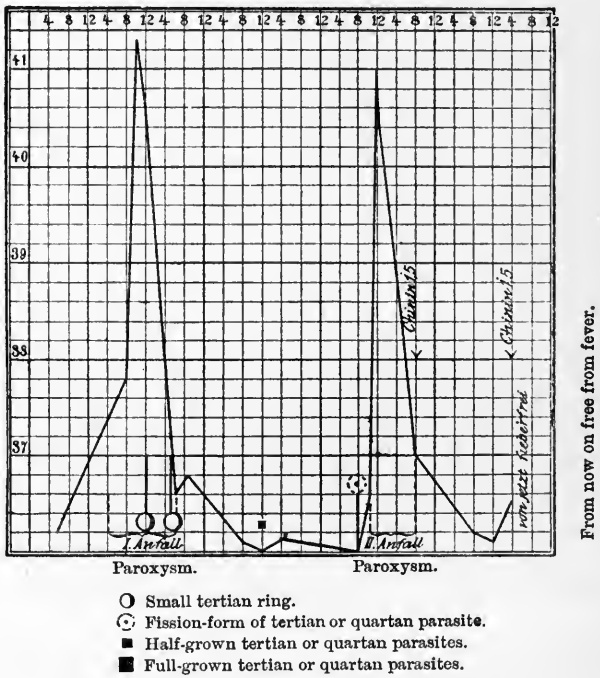
The only objective changes are to be found in the spleen and in the blood. The splenic region is sensitive to pressure, the point of the spleen can be felt projecting beneath the thoracic margin, the splenic dulness on percussion is somewhat enlarged. In lengthy attacks of fever the spleen may attain enormous dimensions. In the blood we find the malarial parasites.

* If we wish to form an exact idea of the duration of a paroxysm of malarial fever, we must take the temperature at least every two hours. In Temperature-Chart XII., on the fifth day of observation, the steep rise and fall of the curve can be clearly seen, because during the fall the temperature was taken every two hours. The whole paroxysm in this case lasted eight hours.

When the temperature falls, the patient sweats profusely, the headache diminishes, and then ceases entirely. All the other painful subjective sensations similarly disappear, and after the paroxysm the patient feels weary, but otherwise quite well. The much-desired sleep soon overcomes him.

Such is the course of a single paroxysm of a simple intermittent fever. But even in these simple fevers the whole course of the disease may be a very various one. The paroxysms may occur every alternate day, or there may be two days free from fever, alternating with a day

TEMPERATURE-CHART I.



○ Small tertian ring.
⊙ Fission-form of tertian or quartan parasite.
■ Half-grown tertian or quartan parasites.
■ Full-grown tertian or quartan parasites.

SIMPLE TERTIAN FEVER FROM WILHELMSHAVEN.

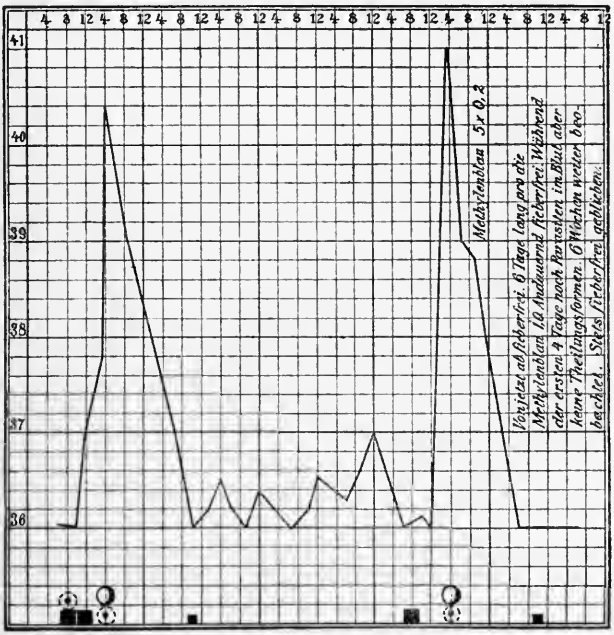
on which there is a paroxysm; or, finally, the paroxysms may occur every day. If the paroxysms occur on alternate days, we have to do with

TERTIAN FEVER.

This fever, like the other type of fever caused by the large species of parasites, has the peculiarity that the paroxysms either recur at exactly the same hour of the day, or else that they come a definite time (one or two hours) earlier—anticipating tertian, or the same amount of time later—postponing tertian. On the days when there is no paroxysm

of fever the patient feels well, and there is only a quite distinctive yellowish-grey pallor, which shows the rapid onset of anæmia. In the absence of suitable treatment, the paroxysms recur for a considerable period, till they finally come to an end spontaneously. During this period, however, the nutrition of the patient obviously suffers. He loses weight, his complexion assumes a greyish-yellow colour, he becomes notably anæmic, his liver and spleen are enlarged, and œdema of the lower extremities frequently occurs, marking the onset of malarial

TEMPERATURE-CHART II.*



SIMPLE QUARTAN FEVER FROM ITALY.

Ill already thirteen months, and has ten times undergone treatment with quinine in various hospitals; of late months the fever has been a pure quartan.

cachexia. Further, in tertian fever there is a great tendency to relapses. The attacks of fever return again and again, until the patient is finally reduced to a most wretched condition.

If, however, the symptoms just described arise in connexion with a fever, the paroxysms of which alternate with two successive afebrile days, we have then to do (see Temperature-Chart II.) with a

QUARTAN FEVER.

To the previous description of the individual paroxysms of fever, and of the general course of the fever, we need merely add that quartan

* For this chart I am indebted to Herr Geheimrath Koch.

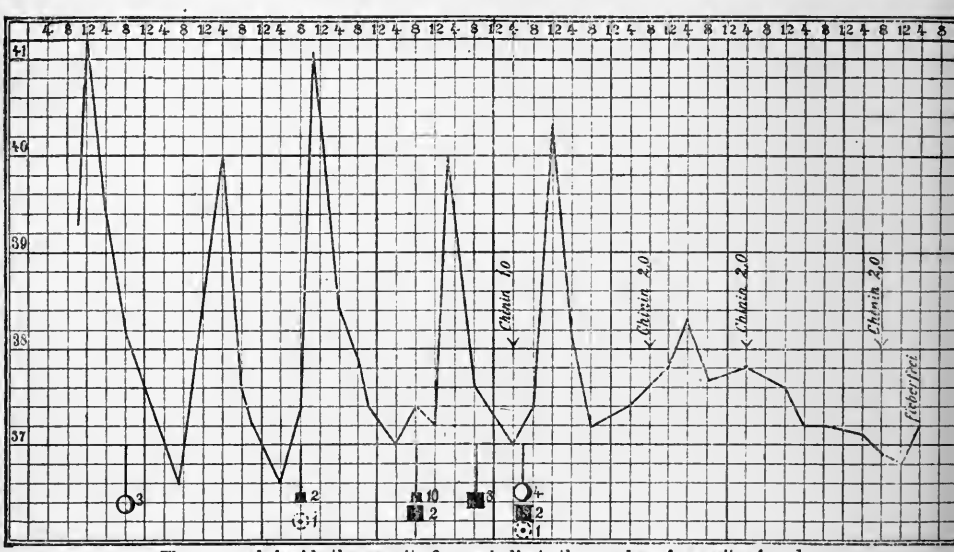
fever is even more obstinate and more prone to relapse than tertian fever, and may afflict a patient for years. This fact was known to the ancient Romans, who, when they wished something really disagreeable to befall anyone, said to him: 'Quartana te teneat!'

It is well known that patients suffering from typical intermittent fever may have daily paroxysms, and fever of this type was, until recently, known as

QUOTIDIAN FEVER,

and was believed to constitute a separate type (*vide* Temperature-Chart III.).

TEMPERATURE-CHART III.



The numerals beside the parasite-figures indicate the number of parasites found.

DOUBLE TERTIAN FEVER FROM THE WEST INDIES.
The appearance is that of a quotidian fever.

This view, as Golgi has shown, is, however, erroneous. These quotidian paroxysms may really result either from tertian or from quartan infection. How this can be brought about will be explained in the section on Pathogenesis.

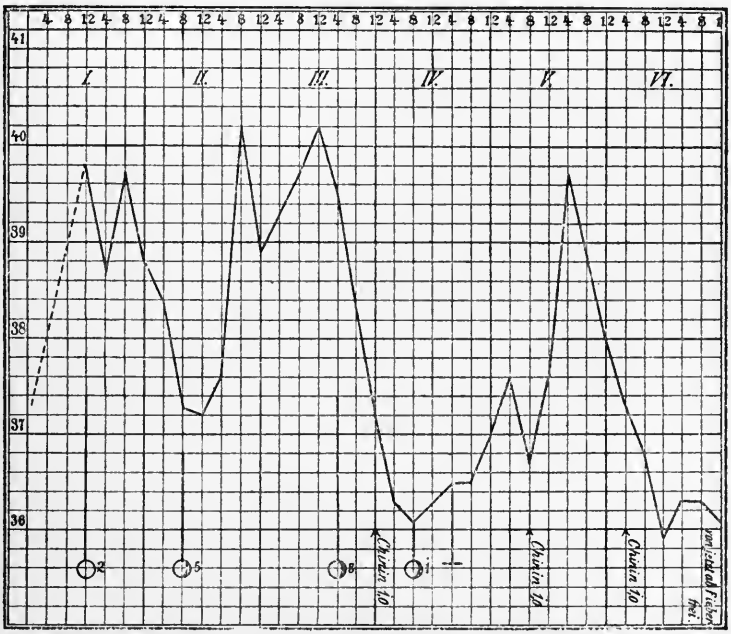
B. Tropical Fevers caused by the Small Species of Parasites.

The curve of this kind of fever was, till recently, unknown. It was supposed that tropical fever was polymorphic, consisting sometimes of a continuous or remittent fever lasting from three days to

three weeks, sometimes of an intermittent fever, and sometimes of fever quite irregular in type. Such a regularity as was observed in the paroxysms caused by infection with the large species of parasites was not to be made out in the case of tropical fevers. It was doubtful whether there was in the latter case any definite type of fever at all.

It is true that the Italian observers had described the curve of a particular kind of tropical fever, the so-called malignant tertian, but

TEMPERATURE-CHART IV.



○ Small tropical ring. ○ Medium-sized tropical ring. ○ Large tropical ring.

TROPICAL FEVER FROM NEW GUINEA.

this curve was believed to be one exceptionally observed, and not regarded as characteristic of tropical fever.

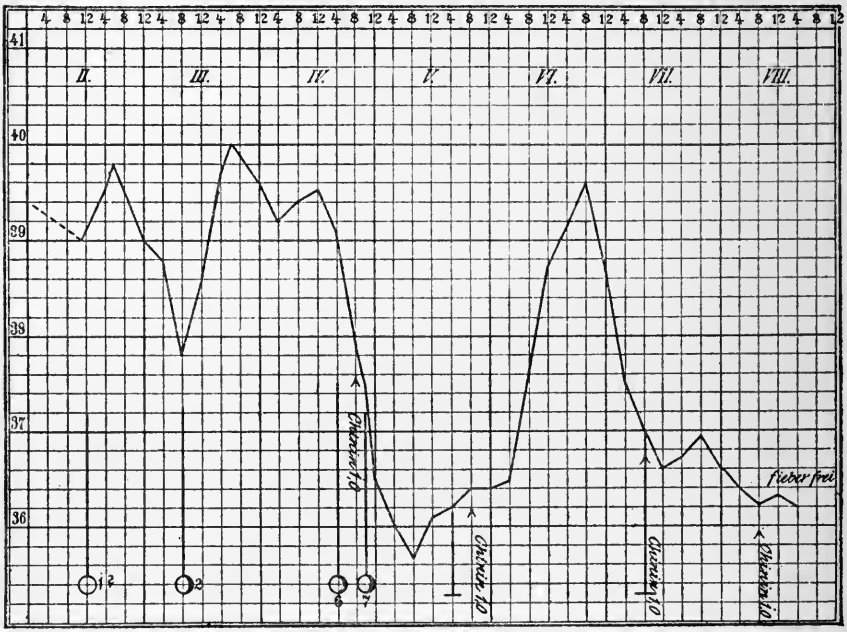
The proof is due to R. Koch that this curve of malignant tertian is not an exceptional phenomenon in tropical fever, but is in truth characteristic of that disease, even though the curve is subject to various modifications. This is a most fruitful discovery, for we are now in a position to diagnose 'tropical fever' from the character of the temperature-curve alone.

As in the intermittent fevers due to the larger species of parasites, so also in tropical fever, there is a prodromal stage, characterized by

a feeling of fatigue, by disinclination to exertion, and by a leaden sensation in the limbs.

The individual paroxysm in tropical fever runs the following course: First of all, the temperature does not rise in quite so abrupt a fashion as in the other intermittent fevers, even though the summit of the rise may be exceedingly steep. The time during which the temperature continues to rise may vary between four hours (*vide* Temperature-Chart IV.) and fourteen hours (*vide* Temperature-Chart VI.). The further course of the paroxysm is also very different

TEMPERATURE-CHART V.



TROPICAL FEVER FROM NEW GUINEA.

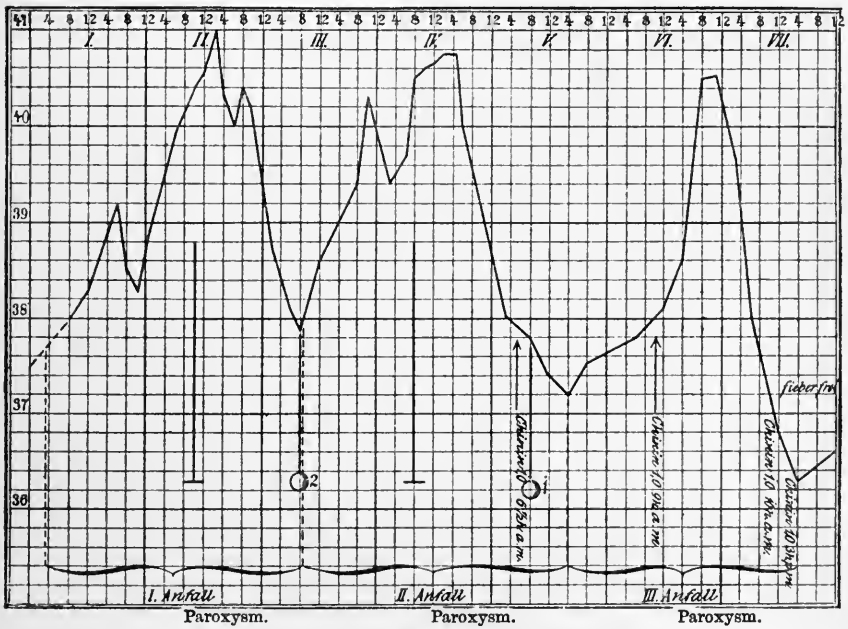
from those previously described. The temperature usually remains high for from twenty to thirty hours, showing during this time either one considerable depression,* or several slight ones, but without falling to the normal. In exceptional cases the duration of the whole paroxysm is from twenty to twenty-four hours only (*vide* Temperature-Chart XVII.). Towards the end of the paroxysm the temperature falls rather sharply, reaching the normal in from twelve to fourteen hours, so that the whole paroxysm may last from twenty to thirty-six hours

* The so-called pseudo-critical depression.

(*vide* Temperature-Charts VI., VII., and XVII.). It frequently happens that the temperature falls only to 37·8° C. (100° F.) (*vide* Temperature-Chart V.), and that even this period of partial apyrexia is quite a short one. In many cases it lasts a few hours only, the fever then coming on once more.

Often the paroxysms follow one another even more rapidly than this. In such cases we no longer have intermissions, but remissions only. The temperature falls only to 38·0° C. (100·4° F.) (*vide* Temperature-Chart VI.), or to 38·5° C. (101·3° F.) (*vide* Temperature-Chart VII.), or even only to 39° C. (102·2° F.) (*vide* Temperature-Chart VIII.).

TEMPERATURE-CHART VI.



TROPICAL FEVER FROM GERMAN EAST AFRICA.

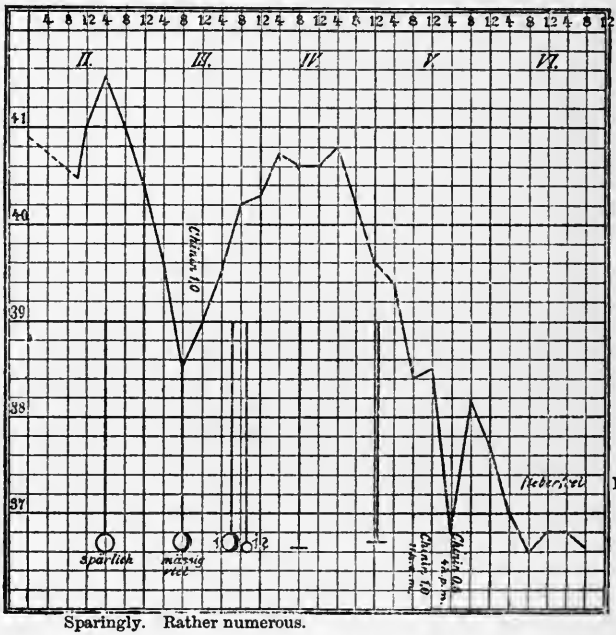
In the latter event the curve for a short period resembles that of a continued fever. But even in these cases the paroxysms can be distinguished from one another, for examination of the blood at once gives us information whether a paroxysm has passed away or is in progress.

In the absence of treatment, the paroxysms recur in the manner just described, but become gradually shorter and less severe, while the afebrile periods become longer in duration. Finally, the paroxysms either cease altogether, or the patient suddenly succumbs in one of them.

I must once more lay stress upon the fact that both the typical curve of the individual paroxysm and also the typical course of the fever as a whole, are, as a rule, to be seen in recently-infected patients only. They are not to be observed in relapses, nor in attacks treated by quinine. A typical curve *may*, indeed, be seen in a relapse, but this is altogether exceptional.

I append a series of charts of tropical fever to illustrate the description just given. From these it is easy to make out that tropical fever always consists of a succession of clearly separated paroxysms, as was first established by R. Koch. The charts given are

TEMPERATURE-CHART VII.



TROPICAL FEVER FROM GERMAN EAST AFRICA.

taken from the most diverse regions. They are all first attacks of fever which occurred on board vessels of the German Navy.

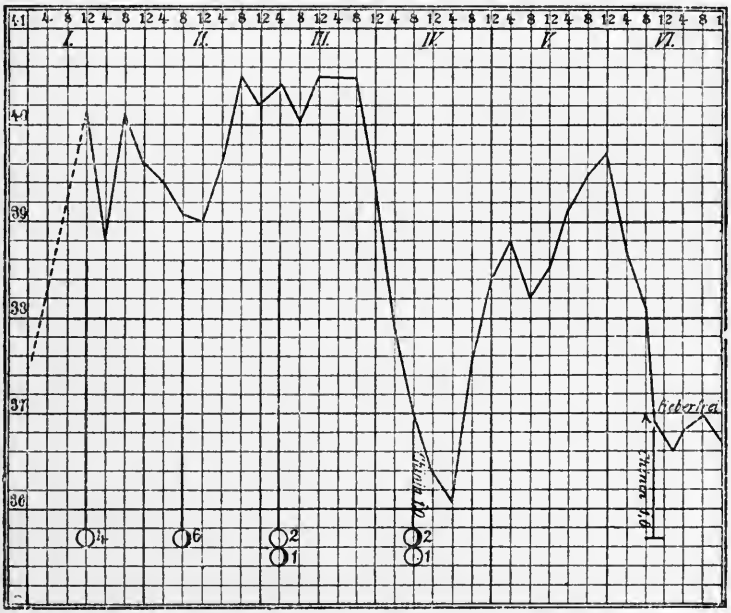
Temperature-Chart IV.: First attack of tropical fever from New Guinea, showing a typical curve in the individual paroxysms. The two first paroxysms are separated by a distinct intermission. Later, the curve is affected by the administration of quinine, and the type of the fever changes at once.

In Temperature-Chart V., a first attack of tropical fever from New Guinea, the two first paroxysms, before the administration of quinine was begun, are separated only by an incomplete intermission

(37·8° C. : 100° F.). But the two paroxysms are none the less quite clearly to be distinguished. After the second paroxysm quinine was given, and the typical curve disappeared.

A similar picture is shown by Temperature-Chart VI., a first attack of tropical fever from German East Africa. Notwithstanding the fact that in this case a remission only to 38° C. (100·4° F.) followed the first paroxysm, the individual paroxysms can be distinguished quite clearly. The case is further noteworthy because it shows clearly how in first infections with tropical fever, notwithstanding very high fever

TEMPERATURE-CHART VIII.



Tropical Fever from New Guinea.

(41° C. : 105·8° F.), parasites may be present in the peripheral blood in exceedingly small numbers.

In Temperature-Chart VII., a first attack of tropical fever from German East Africa, we see paroxysms of fever separated only by a remission to 38·5° C. (101·3° F.). But in this case also the two paroxysms can be distinguished at the first glance.

The curve shown in Temperature-Chart VIII., from a first paroxysm of tropical fever from New Guinea, has at first rather the appearance of a continued fever, but we notice on the second day of the fever a definite fall in the temperature, separating the first from the second paroxysm. The appearance of large tropical rings on the morning of

the second day of the fever shows that the first paroxysm was then just coming to an end.

Naturally, some cases always present themselves in which the fever-curve does not conform to the type described. This will especially be the case when the illness is complicated with influenza, pneumonia, dysentery, or typhoid. Broadly speaking, however, the Charts IV. to VIII. fairly represent the different varieties of tropical fever.

The general symptoms that accompany a first attack of tropical fever are much more severe than those met with in intermittent fever due to the large species of parasites. An exception in this respect, however, is the rigor, which is commonly absent in cases of tropical fever.* The illness is ushered in by a more or less severe feeling of chilliness, and an ill-defined sense of general discomfort. Patients who have previously suffered from fever are so familiar with these premonitory subjective symptoms that they are often able to predict, with considerable exactitude, the approach of a paroxysm of fever half a day or a day in advance. All patients attacked with fever for the first time show the characteristic flushed, hot face, and the burning hot, dry skin. It may happen that the secretion of sweat is suppressed prior to the commencement of the fever. At this time the patients feel fatigued, and have a listless, weary appearance, complaining of giddiness, headache, a feeling of weight in the limbs, loss of appetite, thirst, nausea, and pains in the back and in the bones of the extremities, especially the tibiæ. The most prominent symptom, peculiar to all tropical fevers, is headache, which may become intolerably severe. Another symptom that may cause the patient much suffering is obstinate sleeplessness. Moderate delirium is not uncommon during the height of the fever. The pulse frequency in average cases is about 96 per minute, though it may rise to 130; but the frequency of respiration is, in ordinary cases, very slightly increased. The lips and tongue are dry, the tongue being usually covered with a greyish-white fur; herpes on the lips is seldom seen. In the heart and vascular system generally and also in the respiratory organs there are, as a rule, no morbid changes beyond the increased frequency of pulse and respiration. Occasionally, however, bronchial or intestinal catarrh may appear as complications. In quite exceptional cases the urine contains traces of albumen, but casts are never found.

The spleen, which in first attacks of benign tertian and quartan fevers is almost invariably enlarged, is seldom enlarged at the outset

* A fact already observed and noted by Clark in 1768.

of tropical fever, and by no means invariably so in the later stages of the disease. Enlargement of the liver is very rare in first attacks of tropical fever.

The decline of the fever is sometimes accompanied by sweats, but sometimes this symptom is absent. With the fall in temperature, the subjective symptoms disappear more or less completely.

During the course of a tropical fever—that is to say, as the result of a number of successive paroxysms—somewhat different symptoms may appear.

The most general symptom is the rapid onset of anæmia and emaciation. Even a single paroxysm may make the patient anæmic, and may weaken him to such an extent that he cannot stand or walk without assistance. Since in most cases the patient has one or two paroxysms before quinine is administered, we can easily understand that patients are characterized by a waxy pallor of the face and the visible mucous membranes, and by great debility. In long-standing cases the anæmia may increase to a dangerous degree.

Objectively, the degree of anæmia may be estimated by the use of

Fig. 17*a*.

the Thoma-Zeiss hæmocytometer and of the hæmoglobinometer, and I will therefore describe the use of these instruments.

The Thoma-Zeiss hæmocytometer consists of three parts: a large and wide glass slide, with a very strong cover-glass cemented on to it; a separate and rather thick cover-glass; and a capillary pipette.

1. The cover-glass which is cemented to the middle of the glass slide has in the centre a small, rather deeply-cut circular chamber, on the floor of which is a little table, circular in shape. The upper surface of this table is marked out in minute squares, the side of each measuring $\frac{1}{20}$ of a millimetre, so that the superficial area of each square is $\frac{1}{400}$ of a square millimetre (see Fig. 17*c*). This ruled surface lies exactly $\frac{1}{10}$ of a millimetre below the upper surface of the surrounding wall of the cell (see Fig. 17*a*). Every sixteen squares on the ruled surface are separately marked off by double lines. If the detachable cover-glass be now applied to the top of the cell, we have, between its under surface and the ruled surface just described, a chamber $\frac{1}{10}$ of a millimetre in height. This is known as the enumerating-chamber (see Fig. 17*a*).

The cubic capacity of the space lying between each ruled square and the under surface of the detachable cover-glass is necessarily $\frac{1}{4000}$ of a cubic millimetre.

2. The pipette (Fig. 17*b*) is a capillary tube 10 centimetres in length (*a*), containing an ovoid enlargement (*b*), in the interior of which is a small glass ball (*c*). Beneath the enlargement the capillary tube is marked 0·5 and 1·0; above the enlargement, 101. To fill the tube up to mark 1·0 with blood, a rather large drop of blood is needed. But care must be taken that no blood is sucked in beyond the mark 1, for if this is done it will be necessary to blow the blood out again, to clean the pipette, and begin the operation afresh. Having filled the tube accurately with blood to the 0·5 or the 1·0 mark, the rest of the pipette is filled by suction with normal saline solution to the mark 101, and the two fluids are thoroughly mixed by agitating the glass ball.

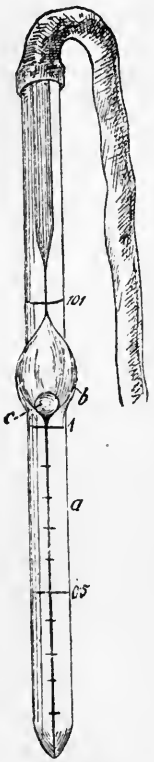


FIG. 17*b*.

A droplet of this mixture is now blown out on to the graduated surface in the enumeration-chamber, and the movable cover-glass is carefully adjusted by sliding it on from one side. This must be done in such a manner that none of the fluid comes to lie between the detachable cover-glass and the sides of the cell on which it rests; and the cover-glass must be pressed so firmly in apposition with these sides as to lead to the formation of Newton's colour-rings. The preparation is now allowed to stand for a few minutes, to allow the blood-corpuscles to sink down on to the ruled glass surface, the microscope is focussed to show the network of squares, and the erythrocytes lying in a number of the squares are counted. To get a trustworthy result we must get an average from counting at least sixteen squares.

If we multiply this average by 4,000, we obtain the number of erythrocytes in a cubic millimetre of diluted fluid. If the pipette was filled with blood to mark 1, we must then multiply the figure so obtained by 100, to obtain the number of corpuscles in a cubic millimetre of blood; if, however, the pipette was filled to mark 0·5 only, we must use 200 as a multiplier.

The mean number of erythrocytes in a cubic millimetre of the blood of a healthy man is five millions.

For the estimation of the hæmoglobin richness of the blood, the best instrument is Gowers' hæmoglobinometer. It is handy, it can be used without artificial light, the results it gives are quite as accurate as those obtained with Fleischl's instrument, and it is much cheaper than the latter.

It consists of two glass tubes of identical bore, fixed vertically side by side in a slab of cork. One of these tubes is sealed at both extremities (*vide* Fig. 18, *A*), and contains the standard coloured solution used for purposes of comparison. The other tube is open at the upper end, and bears a scale ranging from 10 to 140 (*vide* Fig. 18, *B*). A capillary tube (Fig. 18, *C*), with a mark to show the capacity of 20 cubic millimetres, is employed to measure the quantity of blood

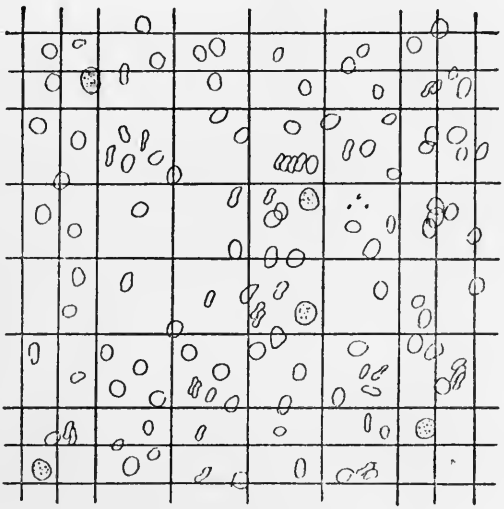


Fig. 17*c*.

required. In addition, a small pipette is required with which water can conveniently be added drop by drop to the blood under investigation.

In using the instrument, we first pour into the graduated tube (*B*) distilled water up to the mark 10. It is not advisable at the outset to pour in more water than this, because the marks on the open tube denote the percentage hæmoglobin content of the blood, and reduction of this content to as low as 15 per cent. has been known to occur.

The finger of the patient is now pricked, and a rather large drop of blood allowed to exude. Care must be exercised, in sucking blood into the capillary tube, to fill it exactly to the mark 20. This blood

is then expelled into the tube *B* containing the distilled water, the point of the capillary tube having first been carefully wiped to remove any excess of blood. On the other hand, the fluid in tube *B*, now consisting of a mixture of blood and distilled water, must again and again be sucked into the capillary tube and re-expelled, so that all the blood in the tube may be washed out and mixed with the distilled water.

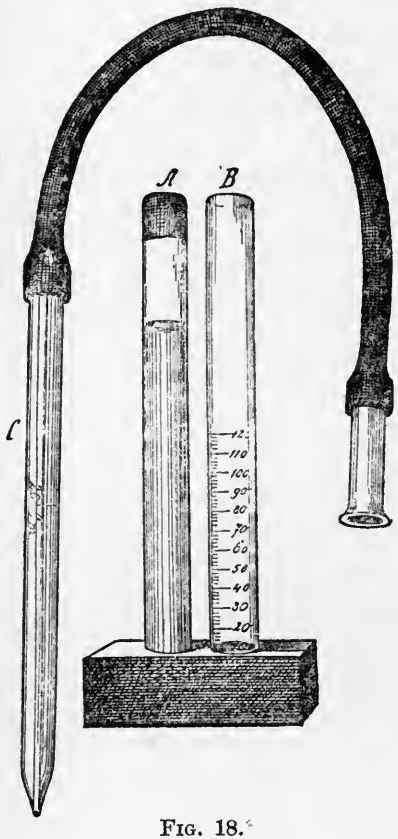


Fig. 18.

Water must now be added to the mixture in tube *B* until the tint of the fluid is identical with that in the standard tube *A*. The mark opposite the upper surface of the fluid in tube *B* gives the percentage hæmoglobin content of the blood under examination.

The hæmoglobin content of normal blood is on this scale represented by the figure 100, so that the percentage given is not the actual content of the blood under examination, but a percentage ratio to normal blood which is taken as 100.

Returning to the description of the symptoms of tropical fever, we must next point out that the heart begins to suffer early in the course of the disease. Weakness of the heart and palpitation are not uncommon. Dilatation and hypertrophy of the right ventricle is frequently observed, but actual cardiac failure is, fortunately, less common. Sympathetic disorder of the digestive apparatus is very frequent, gastro-intestinal catarrh being a very common complication of tropical fever. Even in the absence of this condition, we often meet with uncontrollable vomiting, which may become dangerous to life, the patient being unable to retain any nutriment.

With the symptoms hitherto enumerated, we have by no means exhausted the list of disorders apt to be associated with tropical fever. In former days, especially violent or dangerous symptoms were

regarded as peculiar types of fever. Since, however, all these varieties are due to one and the same cause—viz., the small species of malarial parasites—I consider it erroneous on account of peculiar symptoms to split up into several diseases what is, from the etiological standpoint, a single disease.

Striking symptoms may arise in connection with the nervous system. From the very beginning of the disease, the patients are liable to disorders of consciousness, either lying quietly comatose or manifesting furious delirium. There may be convulsions, and these occur with especial frequency in small children; and mental derangements or partial paralyses may arise. Cases of this nature are variously described in the literature of the subject as comatose, eclamptic, epileptic, cataleptic, and tetanic fever.

It has further been stated that the digestive system is especially liable to sympathetic manifestations of disorder. For instance, cases have been described by various authors in which in every paroxysm of fever dysenteric evacuations occurred, to cease with the cessation of the fever. Thus a special malarial dysentery has been manufactured. But in none of these cases has it been proved that the attacks of dysentery associated with attacks of fever were in truth of a malarial nature. The same may be said of the so-called typho-malarial fever. This is either a tropical fever with rapidly-succeeding paroxysms of fever, or else an attack of typhoid. A true complication of typhoid with malaria is rare. Whether the so-called choleraic form of tropical fever, in which there is no rise of temperature, but which is characterized by profuse evacuations, is really a malarial process appears open to question.

The same must be said of the disorders of the respiratory organs, malarial pneumonia, and malarial pleurisy.

Of the symptoms just enumerated, the most frequent are coma, cardiac failure, and uncontrollable vomiting. Cases in which these complications arise are always to be regarded as dangerous to life.

Blackwater fever (*Fièvre bilieuse hématurique*, *Febris biliosa haemoglobinurica*) occupies a peculiar position. We must first of all distinguish clearly between the manner in which the predisposition to this disease arises and the actual attacks of blackwater fever. The predisposition arises in certain tropical and subtropical regions as a result of repeated attacks of malarial fever, whilst in the great majority of cases the actual attack of blackwater fever is dependent on the administration of quinine.

There has been much controversy concerning this disease. Some observers regarded blackwater fever as the severest form of malaria, and for this reason treated it, not only with quinine, but with quinine in the very largest doses. As much as 12 grammes (3 drachms) has been given in a day (Steudel). As examination of the blood became general, and as in many cases of blackwater fever no malarial parasites were found, physicians gradually abandoned the use of quinine in the treatment of this disease. The first German physicians to abandon the use of quinine, for the reason that they found no malarial parasites in a case of this disease, were Grawitz and Kohlstock (1892). The first, however, really to recognise that in patients suffering from malaria quinine may give rise to blackwater fever was Tomaselli in 1874. Certain Greek authors in 1878, and subsequently F. Plehn in 1895, came to the same conclusion. At the present time it would be difficult to find any ship's surgeon or medical man practising in the tropics who would treat blackwater fever by the administration of quinine. Controversy has, however, arisen as to the real nature of the disease. F. and A. Plehn see in blackwater fever a special kind of malarial process. Sharply opposed to this is the view of R. Koch, who regards the attack of blackwater fever that follows the administration of quinine as quinine-poisoning pure and simple. 'It is,' he writes, 'a quite independent morbid process, *having no direct connexion with malaria*,* but occurring under conditions that harmonize with what we know of hæmoglobinuria in temperate climates. . . . By the pressure of facts, I was gradually forced to abandon my original opinion that blackwater fever is a peculiar modification of malaria, and was led to regard it as a condition due to poisoning.'

What is the clinical course of blackwater fever?

The exciting cause of the disease is almost invariably the administration of quinine.† Either to ward off a threatening paroxysm of fever, or during such a paroxysm, a dose of quinine has been taken. Four hours afterwards the patient has a severe and prolonged rigor, the temperature rising even more abruptly than in a paroxysm of inter-

* I italicize this statement because it has been inferred from Koch's work on Blackwater Fever that this author asserts that there is no connexion at all between malaria and this disease.

† In temperate climates hæmaturia has also been observed to follow the administration of other drugs, such as phenacetin in 15-grain doses, antipyrin, and salicin. It has also followed excessive muscular exertion, exposure to severe cold, or 'a thorough wetting.' Certain inorganic and organic poisons, notably certain poisons of vegetable origin, may also give rise to hæmoglobinuria.

mittent fever. At the same time there is severe headache and long-continued vomiting, which is at times quite uncontrollable. Even small quantities of fluid are immediately rejected. Strength fails with surprising rapidity, jaundice soon comes on, with a small, galloping pulse, often persistent hiccough, which is a very grave symptom; dyspnœa, anxiety and restlessness, and fear of impending death ensue. The patient passes urine of a blackish-red colour, and in some cases also tarry stools. The urine contains albumen, casts, and hæmoglobin in considerable quantities. On boiling, often, the fluid coagulates throughout. After a single attack of blackwater fever the hæmoglobin content of the blood may fall to 25 per cent. As long as the secretion of urine continues fairly free (not less than 200 c.c. (7 ounces) in twenty-four hours), the danger is not extreme; but if suppression of urine comes on, and endures for forty-eight hours, the case almost always terminates fatally. If the illness takes a favourable turn, the urine gradually becomes clear, the objective and subjective symptoms rapidly disappear, and after from two to five days the patient has recovered from the attack, unless, as sometimes happens, the period of convalescence is a lengthy one. This last is especially apt to be the case when the hæmoglobin content of the blood has been very greatly reduced. Malarial parasites are found in a fraction only of the cases of blackwater fever, and, when found, may belong either to the large or the small species.

In other cases, after complete suppression of urine, with continued vomiting, extreme dyspnœa, and terrible anxiety, death ensues from heart-failure.

The illness depends on extensive destruction of the erythrocytes, the hæmoglobin from which, passing into the tubules of the kidney, blocks these, and so brings about the suppression of urine.

Treatment must consist in the free administration of fluids. The vomiting is best relieved by the use of chloroform, from 3 to 5 drops in a little water. Quinine is naturally contra-indicated, even in cases in which malarial parasites are found in the blood, and in its place, when required, methylene-blue* should be used. If this is without effect, we must endeavour, after the blackwater fever has run its

* The only preparation that is of any use is methylene-blue med. pur. Höchst. Inasmuch as it stains of a deep blue colour everything with which it comes in contact, it must be given in gelatin capsules. The dose is from 1½ to 3 grains, as much as 15 grains being given in the twenty-four hours. It should always be combined with a few grains of powdered nutmeg, failing which it is apt to cause painful strangury. The patient must be warned that the urine will be stained blue while the drug is being administered.

course, gradually to accustom the patient once more to the use of quinine, beginning with a dose of 1½ grains, administered by subcutaneous injection. Quinine must, of course, be administered with the same precautions to a patient who, having previously suffered from blackwater fever, is, after the lapse of some weeks or months, once more attacked by malarial fever.

In blackwater fever we have, then, to do with a hæmoglobinuria which is almost always brought about by the administration of quinine. Why it is that only in certain tropical and subtropical countries this idiosyncrasy in regard to quinine is of frequent occurrence is not yet understood. It is probable that the influence of the tropical climate in association with malarial fever leads to the appearance of this idiosyncrasy. R. Koch writes: 'Climate, *per se*, affords no satisfactory explanation why this predisposition should arise, for there are many regions in which tropical fever is prevalent, while blackwater fever is unknown; and, on the other hand, blackwater fever has been known to occur in patients who were suffering, or had suffered, from ordinary benign tertian. If, then, neither to climatic influences alone, nor yet to either of the two known varieties of malaria, we can attribute the origin of this predisposition, we are forced to the conclusion that the etiological factor for which we are looking is, for the most part, at least, a combination of these two elements.'

It is, however, probable that of the two factors it is the malaria that is principally effective, for attacks of blackwater fever in considerable numbers have hitherto been observed in malarious countries only in persons who have already suffered from repeated attacks of malaria. It has never been observed that in tropical or subtropical countries numerous Europeans who had not previously suffered from malarial fever have nevertheless been attacked by blackwater fever.

Especially noteworthy is the fact that the liability to blackwater fever persists long after the patient has left the malarial district, and that even in healthy climates in such persons a dose of quinine may produce an attack of the disease.

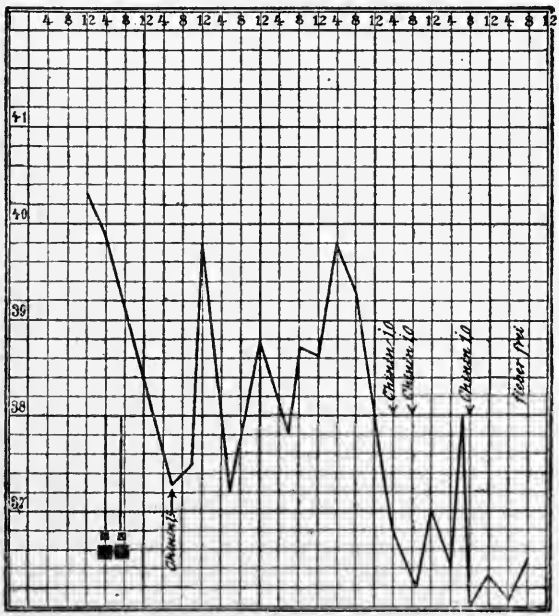
To ward off attacks of blackwater fever, we are advised by R. Koch to attend to the following symptoms: 'If a few hours after the administration of quinine the temperature should rise to 38° C. (100·4° F.) or above, while the urine becomes notably darker in colour, and we observe next morning a slight icteric tinge in the skin, the dose of quinine must be no further increased, for these symptoms

indicate a slight attack of blackwater fever, even in those malarial patients whom there is no other reason to regard as candidates for blackwater fever'—that is to say, even in patients who have not previously suffered from blackwater fever or from especially frequent attacks of malarial fever.

Chronic Malarial Fever and Malarial Cachexia.

Chronic malarial fevers exhibit a less regular and typical course than is seen in recent infections. The fever due to infection with the quartan parasite is the one that retains its typical character

TEMPERATURE-CHART IX.



Chronic Tertian Fever from Cayenne.
About six months' duration.

longest. Furthermore, in the case of quartan fever, the relapses are especially obstinate, and, in the absence of judicious treatment, the attacks may recur during nine months, or even longer. Very gradually, during this period, the quartan type disappears. Fever due to infection with the tertian parasite does not for so long a period retain its characteristic type, the paroxysms assuming an irregular character earlier than in a case of quartan infection. The fever then ceases to have a definite type (see Chart IX.), and it is on blood

examination alone that we can depend for a diagnosis of its nature. It is, however, in tropical fever that the type most rapidly disappears. Frequently in the first relapse we observe that the characteristic temperature-curve has already been lost, and we must avoid drawing any conclusions from the temperature-curve obtained in relapses of tropical fever as to the characteristic course of this disease.

The results of examination of blood in malarial relapses are analogous to the clinical manifestations. It is only in quartan infection that, after numerous relapses, we still find that the development of the parasites in the blood proceeds with perfect regularity. In tertian relapses this regularity no longer obtains, and in these cases we may find parasites apparently on the point of division, and yet no attack may subsequently occur, because, even without the administration of quinine, the further development of the parasite has been arrested.

Finally, in relapses of tropical fever, after repeated attacks, we may find all three varieties of tropical rings simultaneously present in the blood, whilst in other cases we find large tropical rings only. There is, then, no further question of a regular developmental cycle. We must further draw attention to the fact that in relapses spheres and crescents (gametes)—that is to say, the forms destined for further development in anopheles—are present in the blood in greater numbers than in first attacks. The appearance of these forms is the indication of commencing immunization, and in this also we see an explanation of the irregularity in the development of the parasite. This irregularity, however, is due, not only to commencing immunization, but also to the administration of quinine.

The second striking clinical characteristic of chronic malaria is the profound anæmia, which is dependent on the progressive destruction of the erythrocytes. Associated with this anæmia is the enlargement of the spleen, which is more pronounced, and develops more rapidly, in tertian and quartan infection than is the case in tropical fever. The organ may attain very large dimensions, an enlarged spleen sometimes extending downwards to a hand's-breadth below the thoracic margin, and inwards as far as the median line. Enlargement of the liver also is frequently met with in chronic cases. At the same time we usually find dilatation and weakness of the right side of the heart, the patient then suffering from shortness of breath.

If under such conditions quinine be not vigorously administered,

and the patient has no option but to continue to live in a malarial district, we shall observe the gradual onset of the so-called

Malarial Cachexia.

This is merely an extreme degree of chronic malarial infection. Malarial cachexia is differentiated from chronic malaria only by the fact that in malarial cachexia the organic changes initiated by chronic malarial infection are profoundly advanced, and that they often lead to a fatal termination. We therefore find that these patients exhibit the most extreme anæmia, with all the symptoms associated with this condition. They are emaciated, with an ashen-grey complexion, listless, the spleen and liver are enlarged, the heart is feeble, and, as a result, the patients suffer more or less from œdema of the lower extremities and from ascites.

Such a cachectic condition comes on earlier in patients suffering from chronic infection with the small species of parasites than in those whose illness is due to infection with tertian or quartan parasites.

Occasionally in cachectics we find crescents, and even active parasites. Sometimes, however, examination of the blood gives a negative result

The Italian hygienist Celli maintains that immunity from malaria can be acquired only as a result of a fully-developed state of cachexia. This view is, however, erroneous.

It will readily be understood that patients suffering in the way just described are very liable to all other infections. I therefore regard as independent diseases of the nature of secondary infections all those conditions, such as boils, superficial ulcers, gangrene, corneal ulceration, and phlebitis, which have been represented as due to malarial infection.

Larval malaria occupies a peculiar position. This is characterized, in the absence of pyrexia, by periodic attacks of illness, consisting predominantly in disturbances of nervous function. In the intervals between the attacks the patients appear perfectly well. The best-known symptoms of this character are the various neuralgias, and among these, the most frequent is neuralgia of the trigeminal nerve. The branch most often affected is the supra-orbital nerve. In such cases hitherto, in so far as examination of the blood has been undertaken, no malarial parasites have been detected, but none the less these neuralgias are cured by the administration of quinine.

Certain other symptoms have been attributed to larval malaria, such as periodical headache and a sense of debility and depression, which may also recur periodically, without the patient being able to assign any cause for the condition.

Ziemann writes[6]: 'We meet with cases in which at an earlier date the existence of malarial infection has been proved by microscopical examination of the blood, the patients subsequently suffering from repeated, and often serious, disturbances of the general health, usually associated with feelings of deep depression. A radical explanation of such cases is still lacking. Pyrexia is either absent, or is so slight as to be detected with difficulty, and parasites cannot be discovered, notwithstanding the most careful search. And yet the administration of quinine will relieve these most distressing symptoms, which put such narrow limits to the sufferer's working-powers.'

Further, more serious disorders, such as epileptiform seizures, paralyses, hæmorrhages, and even mental alienation, have been attributed to larval malaria. It is true, as we have already seen, that these disorders may arise as the direct consequence of an acute malarial fever; but we must proceed cautiously with our diagnosis when we have to do with a non-febrile condition, and when a long period has elapsed since the last attack of malaria. In the disorders just mentioned the blood has, so far as I know, not hitherto been subjected to examination.

IV. PATHOGENESIS.

A. Tertian and Quartan Fever.

In the description of the malarial parasites given in the first chapter we pointed out that the small forms of the tertian and quartan parasites were found in the blood during the height of the fever, and the fission-forms just before the attack; but the relation between the development of the parasites and the course of the fever was not discussed in detail. The study of this relation must now be undertaken.

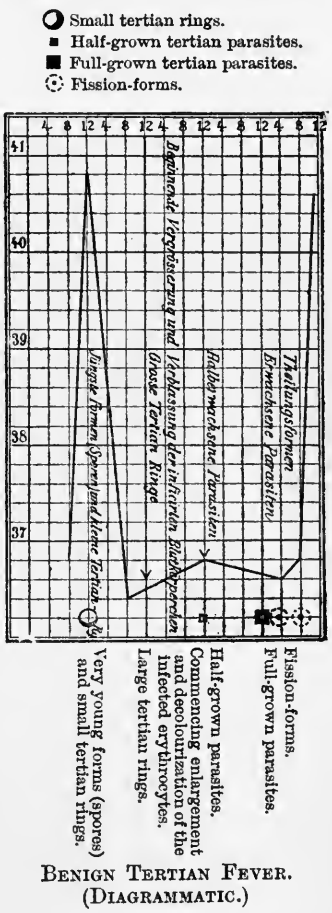
Benign Tertian.

During the height of the fever we find in the blood the youngest forms (spores) and the young forms (small tertian rings) of the tertian parasite. Twenty-four hours after the attack the parasites are half-grown,* often appearing in the form of large tertian rings; thirty-six

* See p. 4 and Plate I., 3.

hours after the attack they almost completely fill the erythrocytes; and, finally, just before the next attack, the fission-forms make their appearance. In these cases the microscopical field shows at any moment identical or very similar parasitic forms. As we pointed out in the first chapter, the individual parasitic forms are not at any particular time of exactly the same size, for, within certain limits, younger and older forms are observed side by side, owing to the fact that the parasites do not all complete their development in precisely the same period of time. The general impression of the picture is, however, not affected by the presence of a few parasites somewhat more advanced in their development. It is naturally during the height of the fever that the most striking differences of form are to be observed, because at this period, beside the numerous very young forms, a few isolated fission-forms are still seen to persist. The presence of these latter is an advantage, enabling us, as it does, to come at once to a definite diagnosis, which would not be possible were the small tertian rings alone present. The relation of the various parasitic forms to the fever-curve is shown in the appended diagrammatic Temperature-Chart X.

TEMPERATURE-CHART X.



BENIGN TERTIAN FEVER.
(DIAGRAMMATIC.)

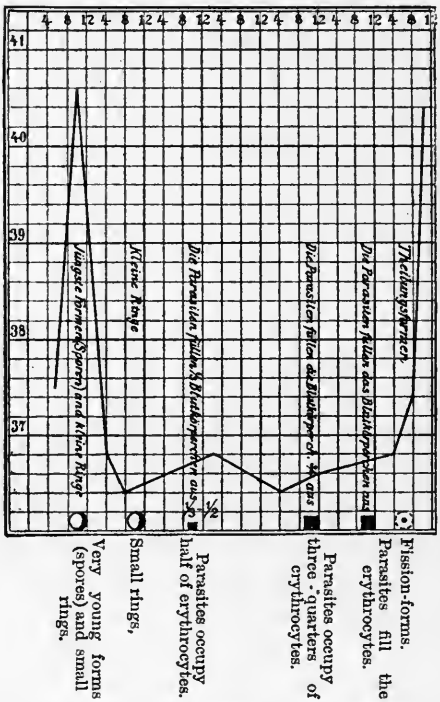
Clinically, one stage in the development of the parasite especially deserves our attention, namely, its fission (sporulation), inasmuch as with the beginning of fission the fever also begins.

We are, therefore, justified in saying that fission (sporulation) of the parasite gives rise to the fever. Whether this is brought about by the setting free in the blood from the erythrocytes, which are destroyed in such large numbers by the process of fission, of a toxic product of the metabolism of the parasites, which previously was stored in the substance of the erythrocytes, or whether the erythrocytic débris

dispersed so largely in the blood has some stimulating effect on the heat-regulating centre, cannot now be determined.

There is, however, another phenomenon seen on microscopical examination of the blood, which has been regarded as explaining the cessation and cure of the fever. For we find, in the neighbourhood of leucocytes that have ingested the pigment set free in the blood by the fission of the parasites, other leucocytes (phagocytes) which have 'eaten' the parasites themselves. It is, however, more than im-

TEMPERATURE-CHART XI.



Benign Quartan Fever (Diagrammatic)

probable that, as some authors allege, this process can bring about a cure. It is probable that processes of a different nature are here at work.

Benign Quartan Fever.

In this we have analogous conditions, conditioned in this case by the development of the quartan parasite.

Here, also, at the height of the fever, we find the very young parasitic forms (spores and small rings) side by side with a few isolated fission-forms. Since the cycle of this parasite is a longer one,

we have, in its case, two days free from fever, during which the parasite is maturing. On the third day, when fission (sporulation) begins, the fever simultaneously appears. The appended Temperature-Chart XI. shows the relation of the individual parasitic forms to the fever-curve.

As far as we have hitherto considered the matter, the pathogenesis of intermittent fever is exceedingly simple. When fission (sporulation) occurs, the fever begins, and when fission is completed, it disappears; whilst during the afebrile period, which, according to the respective terms of development, lasts twenty-four hours in the case of the tertian parasite, and forty-eight hours in the case of the quartan parasite, the parasites are maturing.

Quotidian Fever

has now to be considered, and needs to be explained in a peculiar manner. For a special parasite of quotidian fever has not hitherto been recognised. To put the matter briefly, quotidian fever is either a double tertian, or else a triple quartan fever. What do we mean by this?

(*a*) *Double Tertian Fever.*—By this we mean that there are in the blood two generations of tertian parasites, fission (sporulation) in one generation occurring twenty-four hours after this process has occurred in the other (*vide* Temperature-Chart III.). But since, as we have seen above, fission (sporulation) of the parasites always entails an attack of fever, it follows that if sporulation occurs every twenty-four hours the patient will also be attacked by fever every twenty-four hours—he will have, that is to say, a quotidian fever (*vide* Temperature-Charts III. and III*a*.). To explain in a similar fashion a quotidian fever due to quartan infection, we must suppose that—

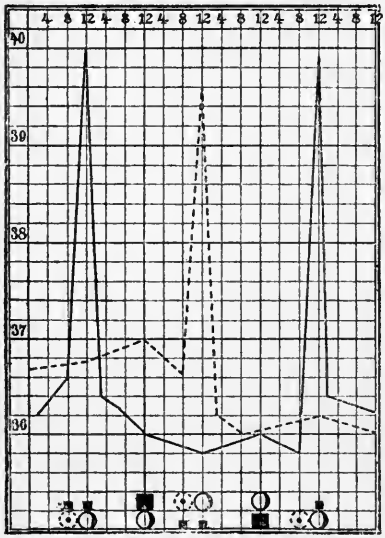
(*b*) Three generations of quartan parasites are simultaneously present in the blood, fission (sporulation) occurring at intervals of twenty-four hours.

And this is in both cases what actually occurs. At the first glance the microscopical appearances in a case of double tertian fever (*Tertiana duplex vel duplicata*), or in a case of triple quartan fever (*Quartana triplicata*), are somewhat perplexing. For all the forms of the parasite, rings, half-grown and full-grown parasites, and fission-forms, are found side by side, so that there seems to be no sign whatever of orderly development. If, however, we make a drawing of what we see, arranging the respective generations of the parasite on the

temperature-chart (*vide* Temperature-Chart III*a*.), we soon observe that the microscopical appearances are in harmony with the explanation. We must naturally bear constantly in mind that the drawings on the charts are diagrammatic, and that owing to the fact that the developmental cycle both of the individual parasites and of the individual generations may vary somewhat in length, certain irregularities may arise.

This is notably the case in Temperature Chart III*a*. According to the systematic development of the parasites, we should, in a case of double tertian fever, expect to find half-grown parasites and fission-forms side by side, or rings and full-grown parasites, but we should not expect in the former case to find also rings, nor in the latter also half-grown parasites; and yet this is what we actually do find. The reason of this is, as already explained, that the development of all the parasites is not simultaneously completed, some isolated individuals maturing earlier, and some later, than the great majority. This is best made out by an examination of the fission-forms. For we find isolated fission-forms even as much as two hours before the attack of fever begins, and, on the other hand, we find others even after the temperature has begun to fall; that is to say, the age of individual members of the same generation of parasites may vary by as much as five or six hours. Since this is once more the case in the second generation, and a difference in age of twelve hours has a very obvious influence on the growth of a tertian parasite, it follows that the parasitic forms met with in the case of a double tertian infection will be more various than appears on the diagram.

TEMPERATURE-CHART III*a*.



I. First generation of parasites.
II. Second generation of parasites.

DOUBLE TERTIAN FEVER (FEBRIS DUPLEX VEL DUPLICATA). (DIAGRAMMATIC).

The appearances found in a case of double or triple quartan are of an analogous character.

We must, however, avoid being led astray by the explanation just given, if, in a case of simple tertian or quartan fever, we find, in

addition to numerous half-grown parasites, a few that are full-grown. We must examine more closely these isolated full-grown parasites, and we shall then find that they are in truth spheres (gametes). These forms, however, are found only after the patient has suffered from several attacks of fever, and when found they are found in all stages of the fever, quite independent of the state of development of the other parasites. Since the spheres have nothing to do with the endogenous cycle of development, we must naturally leave them out of consideration in deciding the question whether one or two generations of parasites are present in the blood.

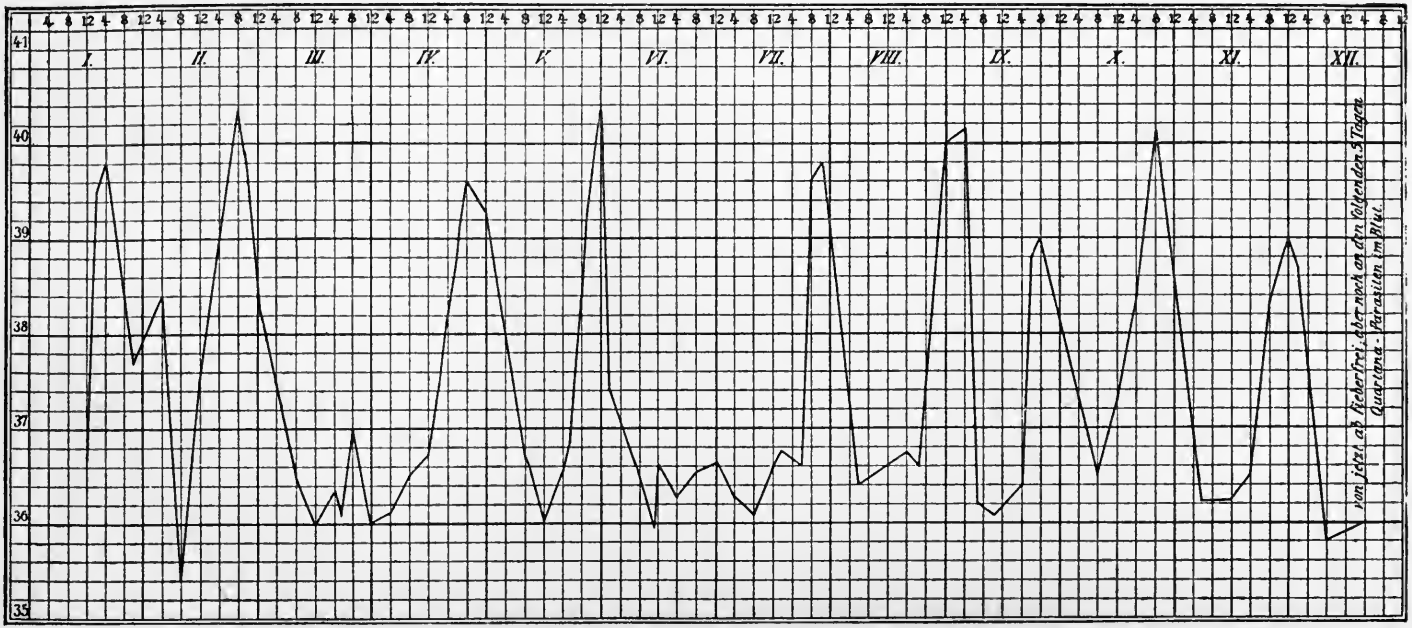
A double quartan fever, one in which two generations only of quartan parasites are found in the blood, is not often observed. In such a case we have attacks of fever on two successive days, the patient on the third day being free from fever. The appended Temperature-Chart XII. (for which I am indebted to Staff-Surgeon Ziemann of the German Navy) shows such a double quartan, passing later into a triple quartan. In this case we must suppose that there were from the first three generations of parasites in the blood, but that one of these contained so small a number of individuals that it was only after a considerable time that they had multiplied sufficiently to cause a paroxysm of fever.

By constructing a diagrammatic chart of a double and triple quartan fever, as has been done in Temperature-Chart XII*a*., and sketching in the stages of development of the respective generations, we readily obtain a general view of the course of such attacks, and of the condition of the blood associated with them.

The explanations already given will enable us to understand without difficulty the transition of a quotidian fever into a tertian or quartan. This phenomenon, which is observed especially in patients who have been removed from unfavourable surroundings and placed under proper care, is brought about by one generation of the tertian, or two generations of the quartan, becoming sterile.

For some time also ' fevers with a prolonged interval ' have played a part in the pathology of malaria. There is no special form of parasite to account for fevers of this character, nor in every case are they to be accounted for only by peculiar irregularities in the development of the parasites. I am of opinion that these fevers in many cases owe their existence entirely to errors of observation. For example, in Temperature-Chart XII., if the temperature had been taken three times a day only—a common practice—the last record being taken daily between four and five in the afternoon, the paroxysms

TEMPERATURE-CHART XII.



From now on free from fever, but on the ensuing five days quartan parasites were found in the blood.

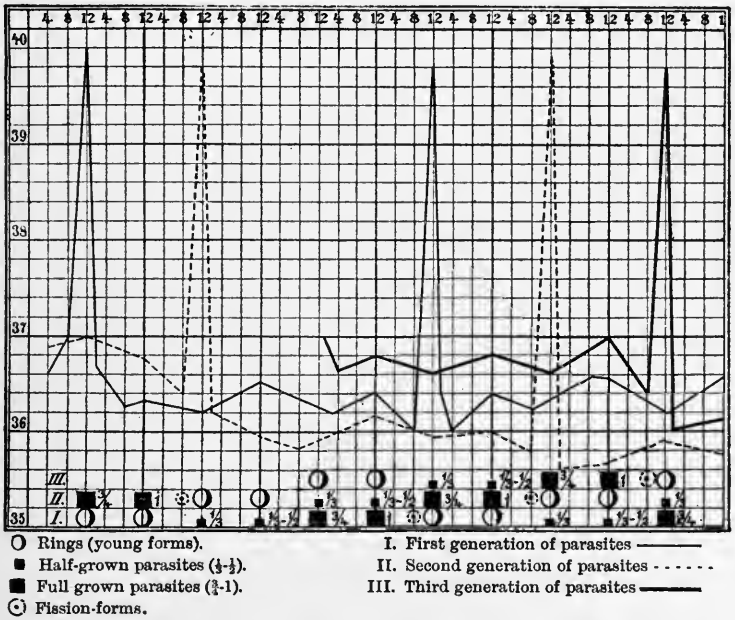
DOUBLE QUARTAN FEVER UNDERGOING TRANSITION INTO TRIPLE QUARTAN FEVER.

that occurred on the second, fifth, eighth, ninth, and eleventh day of observation would have escaped notice entirely, and the case would have been regarded as a simple quartan fever, in which an isolated paroxysm occurred after an ' interval ' of six days.

B. Tropical Fever.

R. Koch not only described the characteristic temperature-curve of tropical fevers, but he also showed the relation between the form of this curve and the development of the small species of parasites,

TEMPERATURE-CHART XII*a*.



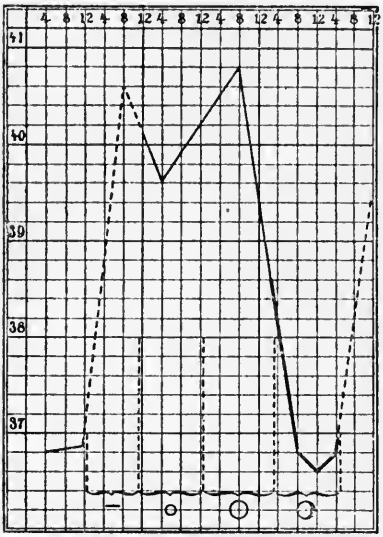
Double and Triple Quartan Fever. (Diagrammatic.)

which is a different one from that which obtains in the intermittent fevers due to infection with the large species of parasites. If we examine the blood while the temperature is rising, and during the first hour or two after it has reached its height, we find in the great majority of cases no parasites at all. It is quite the exception to find parasites at the beginning of an attack of fever; when we do find them it is always in the form of small tropical rings. I intentionally

* Owing to want of space, in those places in which the fission-forms are figured, the other parasitic forms could not be introduced. In order to get a complete picture for these places also, we must add to the fission-forms those forms also that appear on the chart four hours later.

write 'tropical ring' in order to avoid confusion with the small tertian for quartan rings. If we make further examinations of the blood as the fever runs its course, we find, from about the middle of the fastigium to its end, and even on into the middle of the period of defervescence, medium-sized tropical rings—in somewhat greater abundance, indeed, than we find the small tropical rings at an earlier stage, but still sparingly. Towards the end of the period of defervescence large tropical rings begin to be found side by side with the medium-sized rings, and it is not until the apyrexial interval has begun that the large tropical rings are found alone, and then, indeed, in considerable numbers (*vide* Temperature-Chart XIII.).

TEMPERATURE-CHART XIII.



An Attack of Tropical Fever.
(Diagrammatic.)

In Temperature-Chart XIII. the dotted portion of the temperature-curve indicates the period in which no parasites are usually to be found. The period during which they are found in small numbers only is indicated by a thin line, while the period during which they are comparatively numerous is indicated by a thick line.

o = small tropical ring.
○ = medium-sized tropical ring.
◑ = large tropical ring (and also small tertian and quartan rings, *vide* p. 11).
⌣ = crescents.

Isolated specimens of large tropical rings, laggards in development, may be found even during the commencement of the new rise of temperature. But for the most part, at the end of the apyrexial interval they disappear from the peripheral blood. For this reason fission-forms (sporulation-forms) are very rarely to be found in blood taken from the finger. Fission (sporulation) chiefly takes place in the capillaries of the spleen, the liver, the red marrow of bones, and the brain. In the blood of this organ we find very numerous fission-forms.

Whereas in the benign tertian and quartan fevers the parasites

are, as a rule, found in the blood in comparatively large numbers, in tropical fever, even in cases in which the temperature ranges high, and the general symptoms are severe, parasites are during the early part of the paroxysm not to be found at all in the peripheral blood, and later in the paroxysm in very small numbers only; and it is only after defervescence that the large tropical rings appear in comparatively large numbers—that is, in numbers that are large in comparison with the number to be found while the fever is still high.

We should be inclined further to assume *a priori* that in tropical fever, just as is often the case in benign tertian and quartan, two generations of parasites may occur simultaneously in the blood, and that occasionally among first attacks of tropical fever we have to do with a *febris tropica duplicata*. In such a case, not only should all the varieties of tropical rings be simultaneously present in the blood, but also the course of the fever should be a characteristic one.

This, however, as an actual fact, either never occurs at all, or, at any rate, is altogether exceptional.

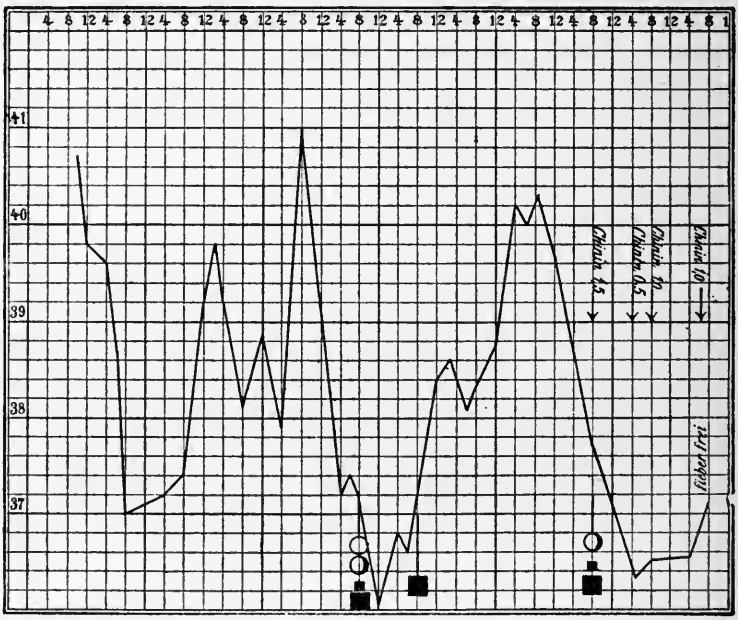
I myself, among the numerous indisputable first attacks of tropical fever in which I have been able to make microscopical examinations of the blood (the cases all occurring on board ship, and the recognition of a first attack being therefore easy), have never succeeded in finding two generations of tropical parasites in the blood, even in cases—which are, indeed, uncommon in first attacks—in which the tropical parasites were present in considerable numbers. Ziemann, on the other hand, has quite recently reported[7] that in the Cameroons he has observed first attacks of fever in which the first paroxysm has continued for forty-eight hours, and in very exceptional cases even for seventy-two hours (*cf.* Temperature-Charts VIII. and XVI*a*.). In these cases, the type of fever, from a clinical point of view, may be regarded as continuous, irregular, or remittent. 'In such attacks,' writes Ziemann, 'we often see in blood from the finger simultaneously all forms of the tropical parasites, from the very smallest rings up to the largest signet rings, with commencing pigmentation.'

On the other hand, a so-called irregular or a so-called remittent tropical fever, has been observed to change into a benign tertian or quartan fever. This also can be explained by examination of the blood. We find in such cases a double infection, either of tropical parasites and benign tertian parasites, or of tropical parasites and benign quartan parasites. Both species of parasites cannot continue to flourish side by side for any long period. Either the small parasites crowd out the large

ones—and this is what usually takes place, as di Mattei proved some time ago by his inoculation experiments—or else the larger species of parasites gain possession of the field. In this latter case the fever assumes the type of a benign tertian or quartan. In such cases, naturally, at the commencement of the attack, the fever has a somewhat irregular character, as is seen in the appended Temperature-Chart XIV., showing a double infection with tropical and benign tertian fever.

The two paroxysms exhibit clearly the characters of tropical fever. In the first paroxysm the whole of which is represented, we see the steep

TEMPERATURE-CHART XIV.



Double Infection with Tropical and Benign Tertian Fever from Eastern Asia.

ascent of benign tertian superimposed on the curve of tropical fever. Since quinine was administered early in the course of the illness, we are unable to say whether, in this particular case, the tropical fever would have been succeeded by a benign tertian.

Every time fission (sporulation) takes place, a large number of the erythrocytes are destroyed, and this destruction, recurring as it does with every paroxysm, explains the extremely rapid onset of anæmia in cases of malarial fever, so that even after a few paroxysms we are struck by the extreme pallor of the visible mucous membranes.

This rapid development of anæmia occurs in all sufferers from intermittent fever, and it is remarkable that its degree has usually no relation whatever to the abundance of parasites in blood drawn from the finger. Even in attacks of fever due to infection with the large species of parasites, the parasites may be found in very small numbers, and yet the clinical manifestations may be quite severe. But such occurrences are much more striking in tropical fever, alike in first attacks and in relapses. In first attacks of tropical fever, characterized by excessive weakness, intense headache, delirium, and a temperature ranging from 40° to 41° C. (104° to 105·8° F.), we may find at the height of the fever one or two medium-sized tropical rings, and during apyrexia five or six large tropical rings (*vide* Temperature-Chart V.). Or it may happen that during the acme of the fever we find no parasites at all, and that it is not until the apyrexial interval that one or two large tropical rings appear (*vide* Temperature-Chart VI.).

We must, therefore, assume in the case of these fevers, either that from the beginning of the attack the parasites accumulate in the internal organs, or else that they are especially virulent.

Contrariwise, it may happen that in first attacks the parasites are found in large numbers in the blood, and yet neither does the temperature rise to any great height, nor are the other objective signs and the subjective symptoms especially severe. In a third class of cases, finally, the condition of the blood, as regards the abundance of parasites and the clinical manifestations, are in harmony. Usually these are attacks in which the appearance of the parasites in great abundance is shortly followed by the death of the patient. This occurs only in cases of tropical fever. In benign tertian and quartan we never meet with such an excessive accumulation of parasites in the blood, nor do we ever find in these infections, as may happen in tropical fever, that several parasites may develop in a single erythrocyte, and that the erythrocytes are infected in a proportion of from 70 to 80 per cent.

To what extent an accumulation of parasites in the capillaries of certain organs may account for the appearance of certain symptoms has not yet been finally determined in all cases. This much, however, may be positively asserted, that an accumulation of parasites in the capillaries of the brain gives rise to coma and to cerebral symptoms in general. For in those who have died in a state of coma the cerebral capillaries have almost invariably been found distended with parasites. But whether the dysenteric symptoms that sometimes occur in connexion with attacks of fever are dependent on an accumulation of

parasites in the capillaries of the intestine is at present as undetermined as the question of the origin of malarial pneumonia. For in this connexion no microscopical examinations have hitherto been made.

In another direction, however, we have advanced farther in our knowledge of the nature of the pathology of malaria, our progress being here once again due to R. Koch. He recognised that the appearance of crescents, concerning the significance of which there has been so much dispute, indicates the commencement of immunization of the patient. For as soon as the malarial parasites can no longer reproduce themselves in the human blood as well as they could at first, the forms destined for the exogenous cycle come into being. These forms are never found during the earlier paroxysms of fever, but only during the later ones; and, indeed, in the case of a first attack, only when no quinine is administered. If, however, in the case of a first attack, the paroxysms are suppressed by quinine, and the use of the drug is then discontinued, crescents appear during the relapse.

With these facts is associated the question whether immunity to malaria can be acquired. The possibility of this has been denied by the English and Italian observers on the ground of their experience of the repeated attacks of malaria from which inhabitants of the Italian and Indian malarial districts suffer. They believe that immunity from malaria can only be brought about by malarial cachexia. R. Koch, however, has shown that immunity to malaria does really arise. He observed among the adult negroes of the East African coast, and also among the adult natives of New Guinea, a well-marked immunity to malaria. The small children of these indigens were not immune, and up to the age of two years were found almost without exception to be infected with malaria. Between the ages of two years and five, it was found that infection was already less common. Immunization, therefore, begins in early childhood.

The apparent contradiction between the statements of the Italian and the English observers, on the one hand, and those of R. Koch on the other, has been explained by the latter in a very simple manner. He shows that those races that possess an acquired immunity to malaria acquired it in very early childhood, in virtue of the fact that the process of immunization was not interfered with by the administration of quinine. Neither in New Guinea nor in East Africa were the children of the aborigines given quinine when suffering from malarial fever. In Italy, in the East Indies, and in Java, however,

matters are on a different footing, the Government in these countries providing for the administration of quinine to the natives. If a process of immunization is interfered with by the administration of a remedy for the disease, immunity is not established. This is also the case in malaria.

If in a community of immune indigens we find an adult suffering from malaria, we must always ascertain whether the patient also is a native of the district in which he has fallen sick, or whether he is an immigrant from a non-malarial region. Until this point has been cleared up we must not, because we find an adult infected with the disease, draw conclusions adverse to the possibility of immunity being acquired in the manner just described.

For example, R. Koch found in Bogadjim, a village in Astrolabe Bay on the coast of New Guinea, a woman of the age of twenty-one years who had malarial parasites in her blood. He ascertained, however, that, notwithstanding the fact that, as a rule, separate villages in New Guinea hold absolutely no communication one with another, in this particular case the woman was an immigrant, and had arrived only six months previously from the non-malarial island of Bili-Bili.

R. Koch further proved a fact no less interesting than important —namely, that immunity to one kind of malaria afforded no protection to the two other varieties of the disease. He found certain islands in which quartan fever only occurred. If inhabitants of these islands were sent as labourers to Stephansort, where tropical fever and benign tertian were prevalent, they sickened of these fevers.

V. PATHOLOGICAL ANATOMY.

We must distinguish between the morbid anatomy of acute and chronic malarial infection respectively. Further, it is to be noted that the descriptions we are about to give relate only to the pathological anatomy of tropical fever, for the reason that benign tertian and quartan are never fatal in the *acute* stages.

In the post-mortem examination in a case of acute malaria, except for more or less developed anæmia, the morbid appearances are not very striking, unless we have to do with a case of icteric blackwater fever. Enlargement of liver and spleen are almost always absent. The spleen, however, is of a chocolate colour, and tears very readily. The liver has a characteristic greyish-brown shade, whilst the grey matter of the brain and the marrow of the bones have a slaty grey appearance.

In the spleen, in such cases, on microscopical examination, we find not only black pigment in very large quantities, but also numerous parasites, especially crescents, and the spheres developed from these latter. The red-marrow of the bones shows similar appearances. In the capillary vessels of the brain are numerous parasites, full-grown or nearly so, in which the pigment is aggregated into a mass. In those who have died in a state of coma, the capillaries of the brain are crammed with pigment and full-grown parasites. In the liver we find brownish-yellow pigment. Everywhere we meet with pigmentiferous leucocytes. Pigment is found in other organs besides the spleen, brain, red-marrow of bones, and liver. But in comparison with the quantity present in the organs just enumerated, the amount found in the other organs is quite inconsiderable.

The post-mortem appearances in chronic malaria are different. In these cases we are impressed first of all by the enlargement of the spleen, which is variable in degree. Occasionally the organ may attain an extraordinary size. The enlargement depends mainly on thickening of the interstitial connective tissue. The liver may show chronic inflammatory changes, or may be already cirrhotic. The kidneys are often atrophic. In all the organs on microscopical examination we find pigment: in the capillaries of the brain, the liver, and the spleen, and above all in those of the red-marrow of bones. Even to the naked eye sections of red-marrow have a dirty grey appearance, and when examined microscopically are seen to contain such large masses of pigment that at first a doubt may readily arise whether what is being examined is dirt or pigment.

As regards the pathological anatomy of blackwater fever, Thin considers that the presence of malarial parasites in the capillaries of the brain is characteristic. In the other organs, in the bodies of those who have died of blackwater fever, malarial parasites are very rarely found. Deposit of pigment in the endothelium of the spleen and the liver may also be absent. The kidneys in blackwater fever are usually somewhat enlarged, the cortex has a light-brown colour, while the pyramids often show a greyish-brown striation; the uriniferous tubules are for the most part plugged with coagulated hæmoglobin or filled with grey tube-casts; the epithelial cells of the tubules are swollen and cloudy, or may have already separated and be in process of disintegration. Hæmorrhages may be found on the pleura and also in the mucous membrane of the stomach and intestines. All the organs of the body have a more or less profound icteric tint.

VI. DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS.

It will easily be understood, from what has already been said, that it is only by the use of the microscope that the diagnosis of malarial fever can be made with absolute certainty. I take this opportunity of laying stress once again on the fact that this certain diagnosis can only be made by the examination of stained preparations. This will be obvious to everyone who recalls how sparingly the small parasites are found in the blood in first attacks of tropical fever, and that this is especially noticeable during the height of the fever. We are often glad in such cases to happen upon a single specimen. If we further bear in mind the minuteness and delicacy of these structures, it will be admitted by everyone who has ever undertaken such investigations that the immature forms of the parasites, when present in the blood in small numbers only, may in fresh preparations very readily be overlooked. On the other hand, an incomplete vacuole, a laceration in an erythrocyte in the vicinity of which the hæmoglobin has undergone some change, or a peculiarly malformed cell, may give rise to erroneous conclusions. With regard to the confusion with blood-plates I will make no comment, since I am of opinion that it is unavailing to attempt the diagnosis, even merely with some approach to certainty, of the so-called spores (young forms) of the malarial parasites when in the free state in fresh blood—unless, indeed, one were to find them still arranged in a group around a mass of pigment.

If, however, in a case of tropical fever, at the height of a paroxysm, we find, even in stained preparations, no parasites, we must wait till the decline of the fever or till the next remission. Then the large tropical rings, which are always more numerous than the small and medium-sized rings, appear. It may, however, happen in a first attack of fever that during the decline of the fever, or in the remission following the first paroxysm, we may find only a single parasite, or perhaps even none at all. This is, however, a very rare occurrence, and has happened to me once only in a hundred cases. Our only recourse, then, is to wait for the second paroxysm, and to make another examination of the blood during the subsequent decline of the fever. We shall now, in a case of tropical fever, always find large tropical rings. We may, however, even then find only isolated specimens. It is well also to examine the leucocytes in the hope of finding some that contain pigment. This is seen far more frequently

during the relapses, and rarely helps us to confirm the diagnosis in first attacks.

In the intermittent fevers due to infection with the large varieties of parasite, the examination of the blood is not attended by such great difficulties. In these cases also, indeed, the parasites may be very few in numbers, but isolated specimens are always to be found, and it never happens that in first attacks in certain stages of the fever they are so completely wanting as occurs in tropical fever. Certainly, if we examine the blood as early as in the prodromal stage (Manson recently described such a case), before definite paroxysms have begun, we may fail entirely to find parasites. It is important to know this, lest in such a case, in consequence of the negative result of our examination of the blood, the diagnosis of malaria should be rejected. Negative also as a rule is the result of examination of the blood in the so-called malarial neuralgias, and in most cases also in the so-called larval malaria.

When the administration of quinine has been begun, in first attacks, the parasites speedily disappear from the peripheral blood, this applying equally to the large and the small species of parasites. The time within which this disappearance takes place is a variable one. In this connexion various circumstances must be taken into consideration. In cases in which isolated specimens only have been found in the blood, the parasite will have disappeared already in two hours after the administration of 1 gramme (15 grains) of quinine, provided the quinine has been thoroughly absorbed. If a gramme (15 grains) of quinine has first been given, and this dose has been repeated in from two to four hours, even though there should subsequently be another paroxysm of fever, the parasites will nevertheless have disappeared from the peripheral blood, and in cases of tropical fever are not to be found in the period of apyrexia that follows this second paroxysm; whereas after a single dose of 1 gramme (15 grains) of quinine, we may often find at this time—that is to say, from thirty-six to forty-eight hours after the administration of the drug—a few isolated parasites. In tropical fever, when a paroxysm follows a single dose of quinine, we never find parasites during the height of the fever, but under these circumstances in benign tertian and quartan a few isolated specimens may often be found.

The results of examination of the blood remain positive for a somewhat longer time in the case of fevers of longer standing. In such cases we may continue to find parasites notwithstanding the administration of quinine, and to find them, indeed, in all stages of development.

From these general considerations it would appear to be fairly easy to establish the diagnosis of 'malarial fever' by means of microscopical examination of the blood. This is, however, only the case when the examination is carried out by a practised observer, who is familiar with the histology and pathology of the blood. Since, therefore, there are many appearances in the blood which are liable to be mistaken for malarial parasites, and since the liability to such an error lays the whole diagnosis open to doubt, it will be well, before describing in detail the mistakes that are liable to occur, to give a short sketch of the histology of the blood. Only after this has been done will certain artifacts be described which are very apt to occur in blood, and often give rise to deception.

The erythrocytes number, according to Ehrlich, in proportion to the leucocytes, from 1,000 to 1 (upper limit) to 360 to 1 (lower limit), and the two kinds of corpuscles are nearly identical in size. Erythrocytes exceeding the average size in the ratio of from $1\frac{1}{2}$ to 2 : 1 are called macrocytes, whilst those notably less than the average size are called microcytes. Erythrocytes that are pear-shaped, or appear as irregularly shaped fragments, are called by Quincke, poikilocytes, and by Ehrlich, schistocytes. Whereas macrocytes and microcytes are of no especial clinical significance, the poikilocytes are found in secondary anæmias. Almost all varieties, when stained by the methylene-blue solution about to be described, assume a yellowish-green or bluish-green tint. (The macrocytes are stained somewhat paler than the others.)

The small number of erythrocytes that stain in a different manner from this become greyish-blue or greyish-green. Since their coloration differs from that of the other erythrocytes, they at once attract the attention. Erythrocytes stained in this anomalous manner are called metachromatic (Ehrlich), whilst those stained in the customary manner are called orthochromatic. If, however, we make use of a mixture of acid and basic anilin dyes—a mixture, for instance, of eosin and methylene-blue, such as Romanowsky's staining solution—the great majority of the erythrocytes are stained a rose-colour. These are the orthochromatic erythrocytes, whilst isolated corpuscles which under the action of Romanowsky's stain assume a reddish-violet or a bright red tint, thus staining in anomalous manner, are in this case called polychromatic, for the reason that the staining solution contains two staining reagents (Ehrlich).

We must be on our guard to avoid mistaking the metachromatic or polychromatic discs for anything other than erythrocytes. Errors

are liable to arise when in badly made preparations the erythrocytes partly overlap one another, and metachromatic or polychromatic discs are, with the exception of a narrow segment, hidden by orthochromatic discs, and thus the uncovered remnants have a falciform shape. In such cases they have been regarded as crescents.

Further, in preparations of blood stained to demonstrate the malarial parasites, we always find erythrocytes containing single or multiple dark-blue granules and stippling. These granules are found also in preparations stained by Romanowsky's method; they have then a tint verging towards green. These imbedded fragments of colouring matter in the substance of erythrocytes were described by Ehrlich as early as 1885, and were regarded by him as indications of degenerative processes in the hæmoglobin. A. Plehn has found them quite recently in 4 per cent. of the blood preparations made by him in the Cameroons; he has called them chromatophil granules, and has described them as the earliest forms of the malarial parasites. This interpretation of their nature is, however, erroneous, and they are, as already said, simply imbedded fragments of staining reagents in degenerated hæmoglobin, which Ehrlich has described as basophil granules, or as the remnants of the original corpuscular nucleus.

But it is not only erythrocytes with nuclear remnants that are found in malarial blood, but also nucleated erythrocytes.

Nucleated erythrocytes of normal size (normoblasts) are found in severe anæmia (also in malarial anæmia), while the large forms of nucleated erythrocytes (megaloblasts or gigantoblasts) are found in idiopathic anæmias. Microblasts or poikiloblasts are very rarely met with.

Far more striking than the differences exhibited by the normal erythrocytes are those exhibited by the leucocytes.

The leucocytes are divided into two groups: first, the lymphocytes (25 per cent.); secondly, the polynuclear corpuscles (75 per cent.), the large mononuclear cells being regarded as predecessors of the polynuclear leucocytes (Ehrlich).

The lymphocytes are as large as or a little larger than the erythrocytes. They contain a large nucleus, staining deeply with methylene-blue, and surrounded by a very narrow margin of protoplasm, which sometimes has a fimbriated appearance. The polynuclear leucocytes contrast strongly with the lymphocytes. They are from two to two and a half times the size of the erythrocytes, possess a lobulated nucleus, which stains deeply with methylene-blue, and have a finely granular protoplasm, which stains neither with acid nor

with basic anilin dyes. Ehrlich has termed this granular protoplasm neutrophil. The so-called eosinophil cells form an exception, their coarsely granular protoplasm staining raspberry-red with eosin. They are formed, according to Ehrlich, only in the bone marrow. and are found in a percentage of from two to ten.

It is probable that the polynuclear leucocytes are derived from the large mononuclear cells, which are from two to two and a half times the size of an erythrocyte, have a strikingly large round nucleus which stains readily with methylene-blue, and have more protoplasm than the lymphocytes. Their protoplasm is neutrophil, and rarely shows any indication of granulation. Besides these mononuclear leucocytes with a round nucleus, we find others the nucleus of which is notched. The deeper this notch, the more distinct is the granulation of the protoplasm, and the more deeply stained is the nucleus.

Yet another structure is found in human blood, the significance of which is not quite clear. These are the blood-platelets (called by Hayem, hæmatoblasts). They are small discs of from one-quarter to one-half the size of an erythrocyte, and stain a uniform greyish-blue to greyish-violet in alkaline solutions of methylene-blue. They are usually found in little groups of from three to ten in number, and they may be found in heaps in malarial blood. We must be careful to avoid mistaking them for free (extra-corpuscular) young malarial parasites of the large species. There are, however, certain criteria to save us from such an error. A malarial parasite of the size of a large blood-platelet already contains pigment, and is never stained so uniformly dull greyish-blue as the latter. The parasite has rather an intense cobalt-blue colour, and shows lighter spots and darker areas. Very young parasites (spores) are more deeply stained at the poles than in the centre, and can thereby be differentiated from the smaller kind of blood-platelets, which have a uniformly greyish-blue colour. In the same way we have to guard against confounding blood-platelets lying on the surface of erythrocytes with the rounded, disc-shaped malarial parasites. The possibility of confounding them with the ring-shaped tropical parasites is, of course, excluded.

But it is not merely those structures that form part of the normal or the pathological histology of the blood which can give rise to error in the examination of blood for the discovery of malarial parasites; there are also certain artifacts which may arise in the making of the blood preparations. So it frequently happens that the natural dimple on the surface of the erythrocyte is much enlarged. In stained preparations the erythrocyte then has the appearance of a narrow rose-

coloured or bluish-green ring, enclosing a colourless centre. Another artifact is found in the vacuoles, which are formed easily if a too thick preparation is dried too rapidly in the flame. In such a case we find that in some particular region of the erythrocyte a circular bright spot appears, the margins of which show sharp and dark contours, while the rest of the erythrocyte is stained in the usual manner. We may also find several vacuoles in a single erythrocyte, and if (as in Plate I., 36) the periphery of these circular vacuoles is stained blue, and a little grain of dirt, as occasionally happens, lies in the circular margin, we may readily mistake these appearances for a ring-shaped parasite. We can, however, avoid this error by examining closely the background of the circular spot. In such pseudo-parasites the space enclosed by the apparent ring is colourless and bright, while the background of the real ring has in its centre exactly the same greenish-yellow or rosy tint as the rest of the erythrocyte. If, however, the deceptive grain of dirt is lacking in the preparation, the distinction is notably easier, as the pseudo-parasite can then be immediately recognised as such, by the absence of the condyloid swelling which is characteristic of the true parasitic ring. The same is the case with those vacuoles which appear in the form of a narrow, bright ring and enclose the normally stained substance of the blood corpuscle (see Plate I., 35). It is, however, also possible that fragments of dirt attached to erythrocytes may be stained in such a manner as to resemble tertian or quartan parasites. In order to establish in such a case a correct diagnosis, we must remember that all large parasites contain pigment. If now these particles of dirt should contain black granules, which might be mistaken for the little rods of pigment, we must consider the form of these rods. The pigment of the parasites has always the shape of little rods or granules, which are fine and delicate. The grains of dirt are always somewhat plump, and have every conceivable shape. In addition to this, we have to examine the whole formation of the object under investigation. The parasites have invariably a structural aspect which is lacking in artifacts. Nevertheless, we must observe that in badly made preparations, in which the erythrocytes have been crushed, torn, or heaped up in thick layers, and in which the parasites have been similarly ill-treated, if these preparations are simply stained with methylene-blue, an absolutely certain recognition of the large parasites, if isolated specimens only are present, is impossible. This is especially, then, the case when, among all other possible contaminations, crushed fragments of the nuclei of leucocytes are superadded, for these remnants

have exactly the same colour as the parasites. In these two last cases Romanowsky's staining method supplies our need, for by it are displayed the red chromatin granules, which are not visible in preparations stained simply with methylene-blue, and these chromatin granules determine the diagnosis; the finer details of the pigment are, however, indistinguishable in preparations stained by Romanowsky's method.

In addition to these artifacts we have certain other appearances to take into consideration, because they also may lead to errors in diagnosis. We find, for example, in the peripheral blood certain leucocytes containing pigment. If these are only about one and a half times the size of an erythrocyte, and have a nucleus which is not lobulated but only notched, the beginner may be doubtful whether or not he has a parasite under observation. Apart from the granular structure of the protoplasm of the leucocytes, which must at once put us on our guard, we find in the leucocytes a deep blue portion, with a sharply defined border, the nucleus, which has no counterpart in malarial parasites, and a faintly stained granular portion, the protoplasm. The pigment lies in the protoplasm.

Inasmuch as malarial parasites do not grow in the leucocytes, we shall find in a leucocyte nothing more than the pigment left in the blood-plasma after the division of the parasite—we shall find, that is to say, lumps of pigment only.

Herein we have a satisfactory criterion for the distinction between pigmentiferous mononuclear leucocytes and spheres (gametes), for the latter are differentiated by the fact that their pigment is scattered throughout their substance in the form of very fine rods. The polynuclear leucocytes may be instantly recognised by their lobulated nuclei. On the other hand, true parasites, the pigment of which is aggregated into a mass, and which are about the size of the mononuclear leucocytes under consideration, show, even in preparations stained simply with methylene-blue, indications of commencing segmentation, which render it impossible to mistake them for any other structures.

Having now described the malarial parasites, and also those appearances that are liable to be mistaken for them, I shall proceed to describe the technique of making blood preparations. A detailed explanation is here imperative, as good preparations are, above all, needed for the successful demonstration of malaria parasites. I advise everyone who, for the first time, undertakes to make preparations of blood, to use glass slides for this purpose. This is easier

than manipulation with cover-glasses, quite apart from the fact that the old method of pulling apart two cover-glasses rarely gave good results, because the structural constituents of the blood were always more or less crushed in the operation. It is of especial importance that this be avoided, as otherwise it may easily happen that the parasites are torn away from the erythrocytes, and are changed to such a degree as to become unrecognisable. Another precaution has to be taken in order to get really good preparations, and that is that the cover-glasses or slides must be quite free from fat. In endeavouring to clean fat from the cover-glasses in the flame of the spirit lamp, it often happens that they crack or bend up at the corners. We prefer, therefore, to use slides, which can be easily passed through the flame without damage.

Hitherto the blood has usually been obtained by pricking the carefully cleansed and disinfected finger-tip with a needle, and by spreading out on a slide the drop of blood so produced. Needle-

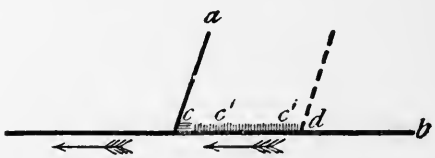


FIG. 19.

a, Top of the inclined cover-glass; *b*, glass slide; *c*, horizontal lines, representing the layer of blood on the lower surface of the cover-glass; *c'*, vertical lines, representing the blood-film on the glass slide; *d*, shows the point on the glass slide where the edge of the cover-glass moistened with blood was first applied.

pricks in the finger-tip are, however, very painful, and therefore the lobe of the ear has often been pricked to secure a drop of blood. Here, on the other hand, the blood is always produced in too small a quantity by a mere prick of the needle; it is therefore necessary before pricking it to massage the lobe a little. I have always found that it is better to prick the back of one of the ungual phalanges than either the finger-tip or the lobe of the ear. The prick in this region is less painful than in the pulp, the skin is thinner, and immediately after the prick a little drop of blood exudes. It may be mentioned also that pricks with small lancets are less painful than needle-pricks.

I have found that the best method of drawing the blood over the slides is that of Jancsó and Rosenberger.[8] The method consists in holding by its upper edge a well-cleaned cover-glass, and moving the lower edge along the drop of blood which exudes from the upper side

of the ungual phalanx, so that the lower edge of the cover-glass is moistened with blood, and at the same time its lower surface becomes covered with blood along a strip of about from 1 to 2 millimetres in breadth. The cover-glass is then placed with the blood-moistened edge downwards upon a glass slide, at an angle of 45°, with the side bearing the narrow strip of blood facing to the right. In this way the strip of blood on the cover-glass is brought into contact with the glass slide. The cover-glass is now quickly pushed from right to left (in the direction of the arrows in Fig. 19) over the glass slide, and the blood is thus spread out without the least pressure.

Preparations made in this manner are fixed in commercial so-called absolute alcohol (96 per cent.), and not in the flame.* They must, however, not remain in the alcohol longer than half an hour. Preparations which have remained in alcohol for an hour or more no longer take stains properly.

Blood preparations are best fixed in the manner usual in the Institute for Infectious Diseases. The cover-glass bearing the blood-smear is grasped with a pair of Cornet's forceps, and two or three drops of a mixture of equal parts of alcohol and ether are allowed to fall on the blood-smear. The preparation is fixed as soon as the mixture has evaporated. In preparations on glass slides two pairs of Cornet's forceps are, of course, needed, and a larger quantity of ether and alcohol mixture. This method renders the use of Petri dishes unnecessary, and is especially to be recommended during travelling and in actual practice.

We now come to the methods of staining, and the simple methylene-blue staining will first be described, as this method is not only sufficient in most cases to enable us to establish the diagnosis, but is also very simple. For recent preparations which have been just dried and fixed the following solution is exclusively used: To 100 c.c. of water we add 0·2 c.c. of caustic soda, and boil the solution. Into the boiling fluid from 0·1 to 0·3 c.c. of methylene-blue med. pur. Höchst is added, and after the solution has been allowed to cool, it is filtered, and is then ready for use. In thin layers it appears violet. A little of the staining-solution is now poured over the blood-smear, and is then immediately washed away with water. The staining-fluid can also be poured into a small glass saucer, in which the preparation to be stained may be held for a few seconds, after which it must be washed

* Fixing with formalin (½ per cent.) cannot be recommended, as the endoglobular parasites in such preparations cannot three months later be stained with methylene-blue, though they can even then be stained by Romanowsky's method.

in water. The preparation appears now to the naked eye of a light violet colour.

By this method the erythrocytes are stained yellowish to bluish green, the ring-shaped malarial parasites dark-blue, the large parasitic forms greyish-blue to deep blue, according to the longer or shorter time during which the staining-fluid has been applied, and the nuclei of the leucocytes appear indigo blue. The basophil granules (stained blue) in the erythrocytes appear just as distinctly differentiated as the metachromatically stained erythrocytes, which are greyish-blue to greyish-green. If we wish to demonstrate these two latter appearances with especial clearness, we must dilute a little of a 1 per cent. solution of methylene-blue of the same degree of alkalinity with ten times the quantity of water, and, having added a few drops to the preparation, we must warm the slide till the fluid begins to steam. The 3 per 1,000 alkaline solution of methylene-blue gains in course of time a greatly enhanced staining power. After three months the staining power of the solution has increased threefold, and it is consequently necessary to dilute it in a corresponding degree before using it. It is also absolutely necessary to filter old solutions before using them.

This method gives, however, good results only in recent, dry preparations of blood, which are not more than four weeks old. Old preparations must be stained with a 1 per cent. solution of methylene-blue, containing 2 parts per 1,000 of caustic soda. Such old preparations must be stained with the greatest care, for even this solution at times over-stains the blood-smears, whereas, on the other hand, the solution has to be allowed to operate for not less than twenty seconds before a sufficient degree of staining is effected. The fact is that old preparations undergo changes of a quite incalculable nature.

In such old preparations it sometimes happens that the blood-plasma itself is stained in certain places, in which case the erythrocytes appear as shining yellow discs on a blue ground. In these bright yellow discs the dark blue or greyish-blue parasites appear sharply differentiated. Preparations stained in this manner are inelegant but convenient, inasmuch as the parasites are strikingly visible.

The most widely known solution of methylene-blue hitherto employed in the staining of blood-preparations is that of Manson (2 per cent. of methylene-blue in a 5 per cent. solution of borax). The staining is, however, so intense that decolourization is necessary.

For this purpose methylal, which has been recommended by Kossel, is the most applicable. The deeply stained preparations are washed in a 2 per cent. solution of methylal (the methylal of commerce is full strength), until the thinnest places are almost colourless. In this manner we obtain preparations similar to those stained by the 3 per 1,000 solution of methylene-blue mentioned above. If no methylal can be procured, some acidulated water—a drop of acetic acid suffices for a tumbler of water—may be used for decolourization.

In this case, however, the parasites are not so well differentiated from their surroundings as in preparations decolourized by methylal. Better results are obtained if Manson's methylene-blue solution is diluted with water to such an extent that the diluted solution in a test-tube becomes slightly transparent. This method is employed in the Institute for Infectious Diseases. The preparations are stained in this diluted solution for from five to ten seconds. The blood-smear then appears to the naked eye of a bluish-green colour, while under the microscope the large parasites appear greyish-blue, the small ones blue-black and the erythrocytes green. The pigment is clearly distinguishable.

Far more beautiful results are obtained by the use of Romanowsky's method of staining, and this method does not only produce elegant preparations, but it greatly simplifies the discovery and recognition of the malarial parasites by the fact that it displays the chromatin by staining it of a brilliant red colour. Romanowsky's method, as originally employed, was very untrustworthy. Ziemann was the first German observer to revive its use, and his modifications made it valuable to a certain degree. Nocht it was who finally discovered the actual staining reagent.

Romanowsky's method consists in the use of a certain mixture of an alkaline aqueous solution of methylene-blue (the methylene-blue medicinale purum Höchst is best adapted for this purpose) with an aqueous solution of eosin. In a successful preparation the malarial parasites appear then of a cobalt-blue colour, with a red grain of chromatin in their interior; the normal erythrocytes are pink; the polychromatic discs reddish-violet or purple; the nuclei of all leucocytes dark violet; the protoplasm of the lymphocytes sky-blue with red stippling; that of the polynuclear leucocytes greyish-red; and the blood-platelets dark violet to blackish-red, while their margins have a fimbriated appearance. This fimbriated margin of the blood-platelets is characteristic, and if attention is paid to this feature, these structures cannot be mistaken for any others. The granules

of the eosinophil cells are stained red, so that the cells appear like raspberries, with violet nuclei.

The reagent which stains the chromatin is the 'red of methylene-blue,' as it has been called by Nocht. This 'red of methylene-blue' separates from alkaline solutions of methylene-blue on heating. It must be present in the solution with which Romanowsky's staining is to be produced. If we wish to determine whether it is or is not present in an alkaline solution of methylene-blue, we have only to shake the solution up with a little chloroform. If the substance in question is present, the chloroform rises to the surface stained the colour of claret. It is, however, remarkable that neither the 'red of methylene-blue' by itself, nor in combination with methylene-blue alone, nor in combination with eosin alone, produces this specific staining of chromatin, but only in combination with both these staining reagents.

The method of preparing the solution is as follows: 0·1 gramme of caustic soda and 1·0 of methylene-blue med. pur. Höchst are dissolved in 100 c.c. of hot, almost boiling, water. The solution is then allowed to cool, and after twenty-four hours 0·2 gramme of caustic soda is added. If this quantity of caustic soda is added in small portions at a time a larger quantity of 'red of methylene-blue' is separated than if the whole of the alkali is added at one time. This solution is heated two or three times during the next twenty-four hours, and is reheated on each occasion shortly before use, but must be allowed to cool before it is actually employed for staining. If a bath of melted paraffin is available, the solution can remain in it for forty-eight hours at a temperature of from 50° to 60° C. (122° to 140° F.). A solution made in this manner has a violet colour.

A solution of eosin of the strength of 1 per cent. is then added drop by drop, while the mixture is repeatedly shaken, to the alkaline solution of methylene-blue, prepared as above described, the addition being continued until a precipitate is formed. This is best done in the following manner:

Ten c.c. of distilled water are poured into an Erlenmeyer's flask, and 1 c.c. of the 1 per cent. solution of methylene-blue is added. In solution thus diluted it is easier to recognise the first appearance of the precipitate than in a more concentrated solution. Drop by drop a 1 per cent. solution of eosin is then added, by means of a pipette, to the fluid in the flask, which is shaken again and again throughout the operation. As this is done, the originally blue solution turns gradually to a brilliant violet, and on its surface a glistening metallic

pellicle is formed. When, amid continual agitation, from 0·3 to 0·6 c.c.* of the 1 per cent. solution of eosin has been added, a precipitate is formed. If the mixture in its present state were employed for staining, we should obtain a useless preparation full of precipitated colouring matter. For this reason mixtures have hitherto been employed for staining in which eosin had been added to a point just short of the formation of a precipitate. To get a satisfactory staining solution, it is, indeed, only necessary to add one-third or one-half of the quantity of eosin solution which would form a precipitate.

1. If we wish to obtain deeply stained preparations, we employ staining solutions containing from $\frac{1}{4}$ to $\frac{1}{10}$ per cent. of methylene-blue.† With such strong solutions of methylene-blue, we need add only one-third of the quantity of eosin solution that would be required to cause precipitation.

Instructions for the Preparation of Romanowsky's Solution of such a Strength.

Into a large flask pour 450 c.c. of distilled water. To get a staining-solution containing a high percentage of methylene-blue, add 50 c.c. of the 1 per cent. alkaline methylene-blue solution, and the flask will then contain a $\frac{1}{10}$ per cent. methylene-blue solution.

Supposing that to produce a precipitate by mixing a 1 per cent. solution of methylene-blue with a 1 per cent. solution of eosin, the ratio between the quantites of the two solutions must be 1·0 : 0·6, or, in other words, that on the addition of 0·6 c.c. of the eosin solution to 1·0 c.c. of the methylene-blue solution, precipitation occurs, we must, if we wish to avoid the production of a stippled appearance in the erythrocytes that are infected with the tertian parasite, add to the 500 c.c. of $\frac{1}{10}$ per cent. methylene-blue solution in the flask only 10 c.c. of the 1 per cent. eosin solution—that is to say, one-third only of the quantity of eosin solution necessary to cause precipitation. The staining-mixture thus obtained will demonstrate the chromatin.

2. To produce delicate tints we employ mixtures containing $\frac{1}{50}$ per cent. of methylene-blue. In this case, however, we must add one-half the quantity of eosin that would be required to cause precipitation.

* Even the methylene-blue med. pur. Höchst is not quite uniform in its staining power. One may always expect some variations within the limits above-mentioned.

† If blood-smears on glass slides are to be stained, at least 500 c.c. of Romanowsky's solution should be prepared, inasmuch as for the staining of such preparations we require from 50 to 75 c.c. of solution in saucers of a diameter of 11 cm.

The stronger solution of methylene-blue is suitable for the staining of old preparations; the weaker solution for recent preparations. Further, the preparations may be stained either rapidly or slowly.

For slow staining, at the ordinary temperature of the room, we employ strong solutions, containing from $\frac{1}{4}$ to $\frac{1}{10}$ per cent. of methylene-blue. From three-quarters of an hour to an hour is then required to complete the staining. The glass slide must be immersed in the staining-fluid with the blood-smear on its under surface, for the reason that, in spite of all possible care, during prolonged staining a fine precipitate invariably forms in the mixture. On removal the blood-smear has a greyish-violet appearance. It must now be decolourized in acidulated water containing 1 drop of acetic acid in a tumblerful of water. Washing in acidulated water serves not only partially to decolourize the specimen, but also to remove the fine precipitate that always forms in preparations stained by Romanowsky's solution.

Preparations stained rapidly in warm diluted solutions of methylene-blue (of a strength of $\frac{1}{50}$ per cent.) have a different appearance.*

Preparations that have been warmed in the staining-solution until the latter just begins to steam—which, when a Bunsen flame is used, takes about fifteen seconds—and have then been allowed to lie in the warm solution for two minutes, the solution being subsequently warmed for about ten seconds longer over the Bunsen flame, until steam is once more given off; and, finally, have lain three or four minutes more in the warm solution—such preparations when taken out appear almost colourless. At the edge only of the slide is there a violet border. These preparations are free from precipitate, and need only be washed in water. When stained in this manner the erythrocytes have under the microcope a dull, greyish-red appearance, whilst the parasites, which are stained a deep cobalt-blue colour, with the chromatin granules tinged purple, are sharply differentiated from the dully stained erythrocytes.†

* If we stain in warm strong solutions of methylene-blue (of a strength of from $\frac{1}{4}$ to $\frac{1}{10}$ per cent.), especially if, as is necessarily the case with old preparations, the immersion lasts as long as fifteen minutes, more or less adhesive precipitates are always formed. Such preparations, after decolourization in acidulated water, must be washed in alcohol. Under this treatment the precipitate disappears while the chromatin staining persists. Whereas in preparations stained in cold solutions, acidulated water suffices to remove the precipitate, in those stained in warm solutions alcohol is needed for this purpose.

† Only the deeply stained preparations are permanent. Those that have been delicately stained lose their colour within three months, even when mounted in cedar-oil or in Canada balsam free from sulphuric acid. Canada balsam containing sulphuric acid bleaches the preparations very rapidly.

If the saucers used to hold the staining-solution are made of metal, they begin to steam within five seconds over the flame. The process in this case is similar to that above described, the heating being intermittent, and the average duration of the immersion of the specimens in the warm solution being six minutes.

Romanowsky's solution must always be freshly made. Further, it must be used once only. A second specimen must never be stained in the same portion of fluid.

A phenomenon must now be described which is always observed in the use of Romanowsky's method, and the nature of which it is important to understand.

During the admixture of the two components of the stronger staining-fluid ($\frac{1}{10}$ to $\frac{1}{4}$ per cent. of methylene-blue), a metallic pellicle, as already mentioned, forms on the surface. This pellicle, the formation of which is an indication that the solution has attained the strength requisite for the staining of chromatin, does not make its appearance in the weaker staining-fluid ($\frac{1}{50}$ per cent. of methylene-blue) until the mixture is warmed. If, however, the pellicle fails to appear on warming the fluid, this is an indication that some mistake has been made in the admixture, and such a solution is useless, for the chromatin will remain unstained.

To ascertain if the staining has been successful, we first examine the specimen under a low power (Leitz obj., No. 3). If the nuclei of the leucocytes are then seen to be stained violet or red, we know that the chromatin has also been stained, and the preparation may be mounted in oil for further examination.

Romanowsky's staining method may also be used to a certain extent in the differential diagnosis of the species of malarial parasites.

If to the methylene-blue solution we add from one-half to two-thirds* of the quantity of eosin needed to cause precipitation, and stain the preparation with the warmed solution in the manner above described, there is produced in erythrocytes infected with the tertian parasite a quite characteristic stippling.† The degree of stippling is directly proportional to the age of the parasite. The stippling may either resemble eosinophil granulation, or may be blackish-red in colour. Unfortunately, in the case of the small and very small tertian rings

* We must not add more than two-thirds, for the reason that in this case, when staining with the stronger methylene-blue solutions, precipitates are formed which cannot be removed by the use of alcohol.

† I described this stippling in the *Zeitschr. f. Hygiene u. Infektkr.*, 1900, p. 33, and my observations were shortly afterwards confirmed by Maurer.

this stippling is apparent only in erythrocytes containing two rings; when the erythrocyte is infected by a single parasite only, the stippling does not appear until the parasite is from twelve to eighteen hours old.

In the case of quartan and tropical parasites, on the other hand, this stippled appearance is never produced.

To demonstrate the stippling, we stain fresh, dry preparations in solutions containing $\frac{1}{50}$ per cent. of methylene-blue; older preparations in solutions containing from $\frac{1}{4}$ to $\frac{1}{10}$ per cent.

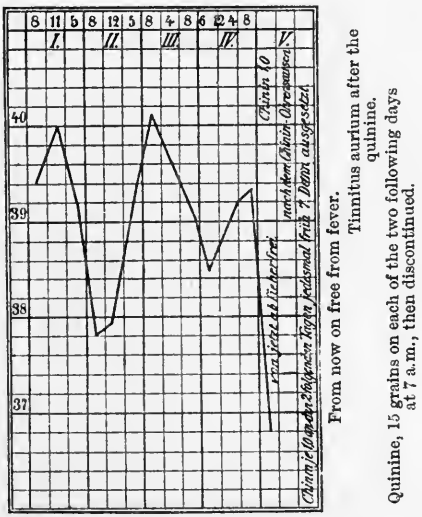
Greatly simplified as Romanowsky's staining method now is, it is none the less a far more lengthy process than the never-failing simple staining with methylene-blue. I therefore advise that for general use, and in staining simply for diagnostic purposes, this latter method should be employed, and that Romanowsky's method should be reserved for preparations containing one or two parasites only, so that their presence cannot be detected with certainty by simple staining with methylene-blue. Romanowsky's method is also of especial value in cases in which the preparation has been badly soiled, or the erythrocytes have not been evenly spread out, and the parasites have been injured in the process. In all these cases the demonstration of the chromatin-granules by Romanowsky's method establishes the diagnosis with certainty.

In the great majority of cases, however, we can also, by simple clinical observation, determine with considerable approach to certainty the diagnosis of malaria. In the intermittent fevers due to infection with the large species of parasites, the abrupt rise and the equally abrupt fall of the temperature-curve have long been recognised; and when attacks of fever with a curve of such characters recur at the same hour every third or every fourth day, with the typical sequence of rigor, heat, and sweating, while in the intervals the patient feels well, and a moderate swelling of the spleen is associated with the rapid development of anæmia, the diagnosis of intermittent fever is made with certainty. The same confidence is felt as to the nature of the disease when fever with the above-mentioned accompaniments recurs daily, and is duly controlled by the administration of quinine. But cases of daily recurrent fever always remain, as will be shown later by specific examples, in which doubts may arise as to the nature of the disease.

It is quite otherwise in the case of tropical malaria, in which diagnosis based on clinical observation only is far more difficult. As

we saw above, the temperature-curve of tropical fever has long been recognised, but was believed to be characteristic of only a very small number of cases of fever. R. Koch was the first to establish the fact that the temperature-curve of malignant tertian is, in fact, the characteristic curve of tropical fever. The features of this curve are so unmistakable that in a great number of cases from it alone the diagnosis can be established. It must, however, not have been affected by the administration of quinine, and, further, the case must not be a relapse of tropical fever; moreover, we shall often have to wait from thirty-six to forty-eight hours or more before we can make our diagnosis, because only after this lapse of time as a rule has the

TEMPERATURE-CHART XV.



TROPICAL FEVER, WITH AN IMPROPERLY DRAWN TEMPERATURE-CURVE.

first paroxysm passed away and the second begun. Further, it may happen that the attacks run into one another, so that apyrexia is not clearly manifested (see Temperature-Charts V., VII., and VIII.). Still, even in these cases we can recognise the individual paroxysms.

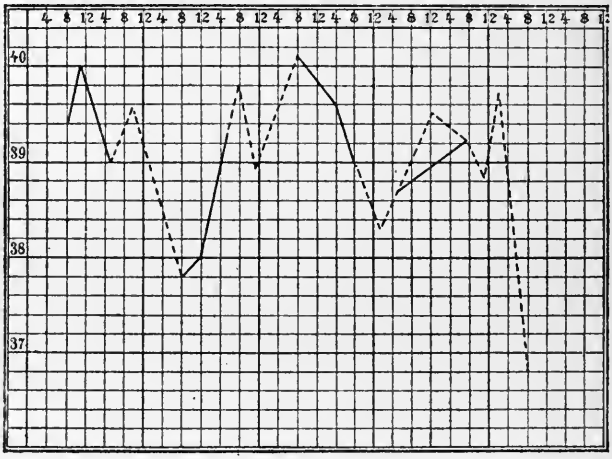
We can no longer speak, as was formerly done, of a complete irregularity of the temperature-curve in tropical malaria.

How it came to pass that the typical temperature-curve of tropical fever so long escaped recognition must now be explained.

In the first place, a very large number of febrile disorders were formerly regarded as malarial in nature, which, as a matter of fact, had nothing whatever to do with that disease. Typhoid especially,

the existence of which in the tropics was at one time totally denied by many authors, was regarded as malarial fever. Hence arose the belief that tropical fever may have the type of a remittent in which the fever continues for two or three weeks. Since, moreover, both tertian and quartan fever also occur in the tropics, and may appear in the form of double tertian or triple quartan, as the case may be—apparently, that is to say, in the form of a quotidian fever—the conclusion was naturally drawn, prior to the discovery of the malarial parasites, that tropical fever might also have a quotidian type. But inasmuch as no distinction was made between first infections and relapses of tropical fever, and in consequence of this, even in true tropical fever, the temperature-curve was regarded as quite irregular, it appeared also that the course of the disease was altogether protean. It further

TEMPERATURE-CHART XV*a*.

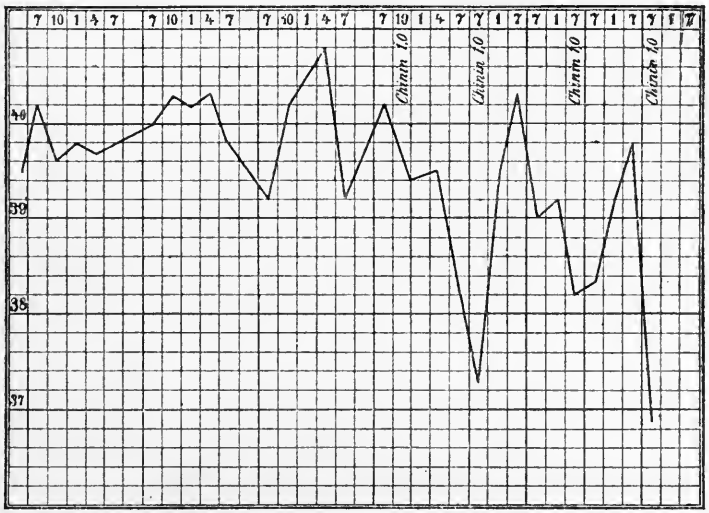


happened, first, that the course of these fevers was almost invariably disturbed by the administration of quinine; and, secondly, that the temperature-curves of the individual paroxysms were improperly drawn, for the reason that the temperature was not taken during the night. Hence arose a hiatus in the observations, which was not sufficiently taken into account.

The mistake that arises from the failure to recognise this hiatus can be readily understood from the examination of temperature-curves that are in other respects correctly drawn, on which the hours at which the temperature was taken are inscribed. I shall proceed to give some examples of this nature.

Temperature-Chart XV. represents an irregular, partly intermittent, partly remittent fever. From the second to the fifth day the curve has the character of a pseudo-tropical fever. I call it pseudo-tropical because paroxysms of sixty-eight hours' duration are not met with in tropical fever. In Temperature-Chart XV*a*. the curve of Chart XV. has been reconstructed. What was actually measured is shown by the continuous line, and the probable course of the fever in the unrecorded intervals is shown by the dotted line. This curve shows not only the importance of taking the temperature at regular intervals, which should not exceed four hours, but also of an accurate record of the observations. In Temperature-Chart XV., for instance, intervals of three hours are indicated on the chart by the same space as that

TEMPERATURE-CHART XVI.



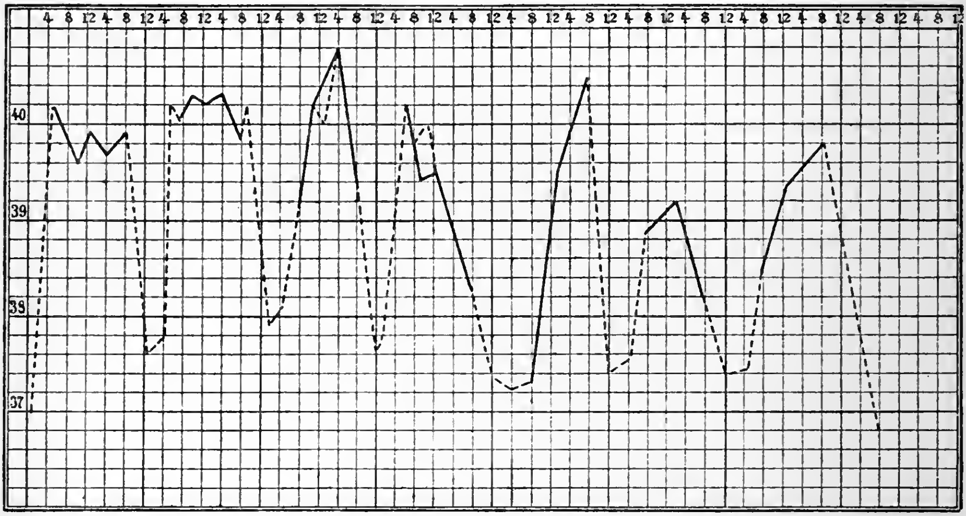
Tropical Fever, with improperly constructed Curve.

given to intervals of six and of fifteen hours. The character of the curve is thus entirely falsified, and we are unable to learn from it that, for example, the second paroxysm began quite typically in the night between the second and the third day of observation. Owing to the erroneous construction of the curve, only half of this paroxysm is represented.

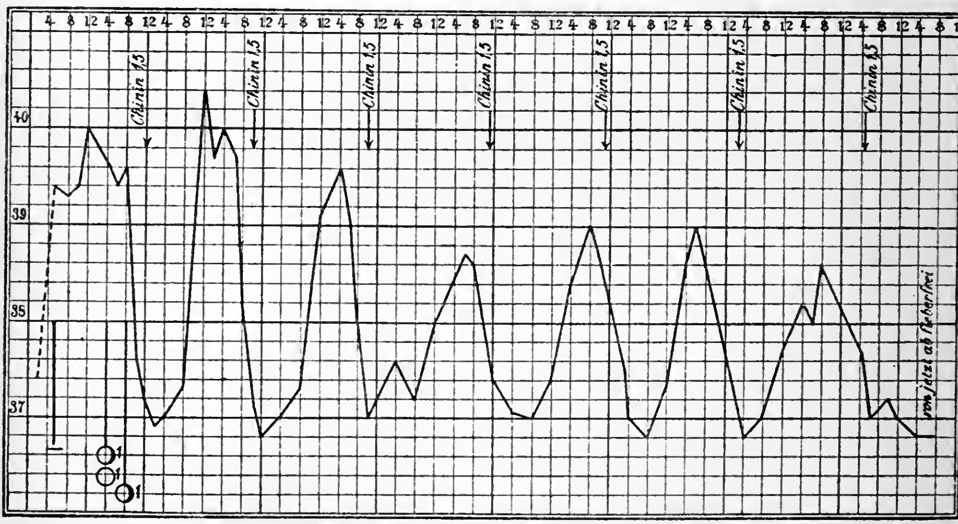
Temperature-Chart XVI. shows similar errors. The construction of the curve is less faulty than that in Temperature-Chart XV., but in this case also no observations have been made at night. In Temperature-Chart XVI*a*. we see what has probably been the real nature of an apparently continuous fever passing into irregular fever.

The reconstruction of these curves may, however, be regarded as arbitrary, and this is especially the case with Temperature-Chart XVI*a*.

TEMPERATURE-CHART XVI*a*.



TEMPERATURE-CHART XVII.



From now on free from fever.

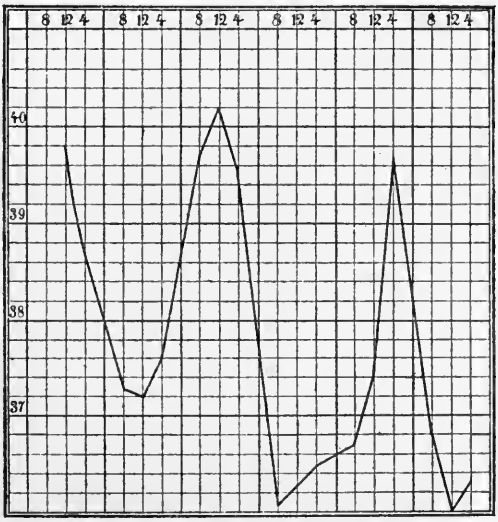
TROPICAL FEVER (FIRST INFECTION) FROM GERMAN EAST AFRICA.
The numbers adjacent to the rings show the number of parasites found.

I therefore show in Temperature-Chart XVII. an actual chart of tropical fever, which, notwithstanding the administration of quinine, has characters identical with those of the reconstructed curve XVI*a*.

I am of opinion that in the case of Temperature-Chart XVII. the quinine has not only lessened the duration of the individual paroxysms, but has also increased the depression of temperature in the intermissions, and has lengthened the apyrexial periods, since in the first paroxysm the temperature has already risen high at six o'clock, whereas in all the succeeding paroxysms it does not begin to rise until eight o'clock in the morning.

Conversely I shall now show what may become of typical curves in consequence of insufficiently frequent records of temperature and erroneous construction. I have continued to preserve regular intervals between the daily records, three per diem, and have reduced the

TEMPERATURE-CHART XVIII.

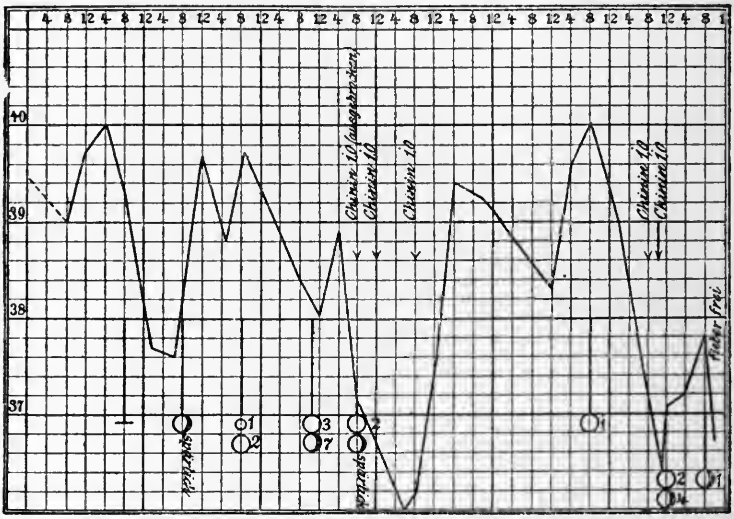


nocturnal interval to one-half only, whereas in improperly drawn curves this reduction frequently amounts to one-third. The typical tropical fever curve of Temperature-Chart IV. is transformed into a simple, somewhat long-drawn-out tertian (see Temperature-Chart XVIII.), and that of Temperature-Chart XIX. into an irregular remittent (see Temperature-Chart XIX*a*.), which later, as was formerly said, passed into an irregular quotidian. The remittent fever of the first days is produced by the fact that the intermission to 37·6° C. (99·7° F.), which occurred on the morning of the second day between two and six o'clock, is not represented, owing to insufficiently frequent observations, and is converted into a remission.

Even more striking is the difference between Temperature-Chart XX.* and Temperature-Chart XX*a*. The former shows a long-

TEMPERATURE-CHART XIX.

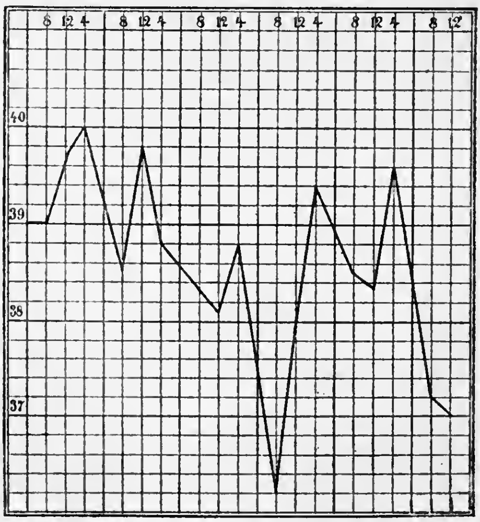
Vomited.



Sparingly. Sparingly.

Tropical Fever, complicated with Dysentery (First Infection), from German East Africa.

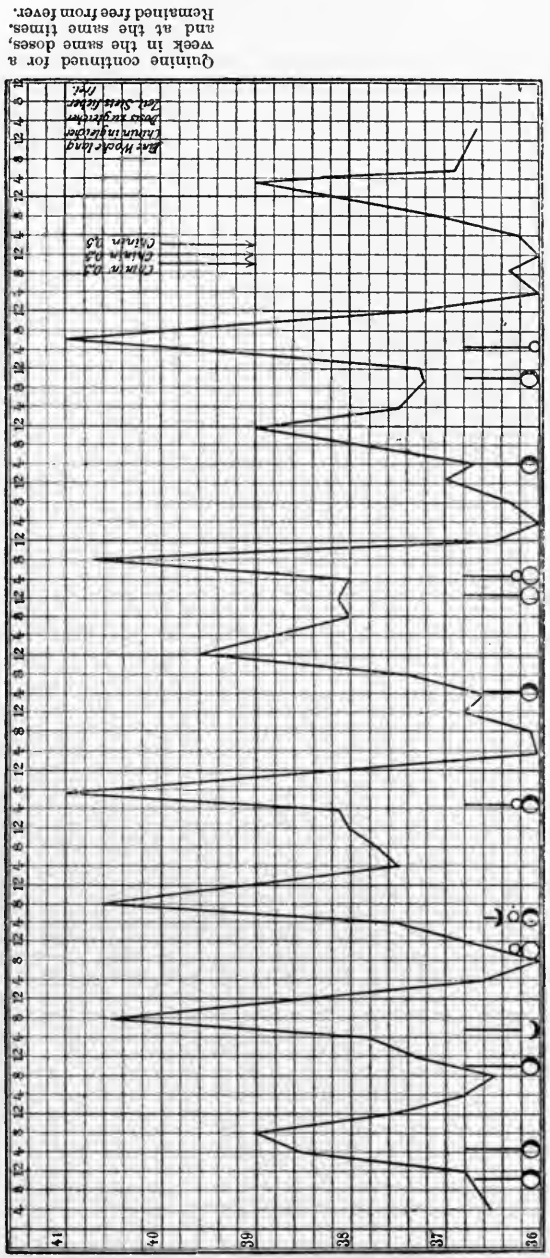
TEMPERATURE-CHART XIX*a*.



continued Italian summer-autumn fever (tropical fever) in which crescents were found in the blood on the second day. The curve is

* I am indebted for this chart to Privy Councillor Koch.

remarkable for the depth of the pseudo-critical depressions and for the sharp-pointed summits. None the less the tropical fever consisting of a number of successive paroxysms can clearly be recognised.



TEMPERATURE-CHART XX.

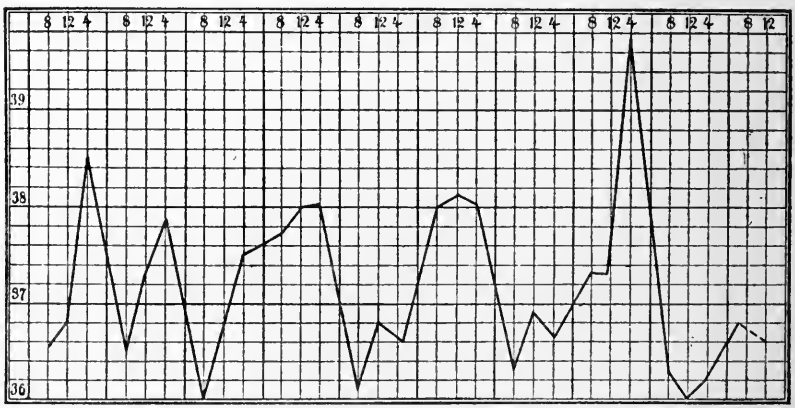
SUMMER-AUTUMN FEVER FROM ITALY.

If, however, no records are taken during the night, and the nocturnal intervals are, as has hitherto been the usual practice, com-

pressed to one-half of their true size, we get a curve which is represented in Temperature-Chart XX*a*., one which in the malarial pathology of former days was described as subcontinuous.

If, therefore, we wish to demonstrate the typical curve of tropical fever, we must take the temperature at least every four hours, and continue the observations throughout the night. Only in this way can the errors just indicated be avoided. The best charts for recording the observations are those made by R. Koch. On these the curve must always be accurately drawn, even if a particular observation is wanting, or if the observations have been taken at irregular intervals, as may readily happen when the physician has to carry on the observations single-handed. In such cases the physician will do well to combine examinations of the blood with measurements of the tem-

TEMPERATURE-CHART XX*a*.



perature. In this manner he will not only most rapidly form his diagnosis, but will also discover the time most favourable for the administration of quinine. The fever will then be more rapidly reduced, he himself will be saved much time and labour, the patient will be spared many dangers, and the number of patients to be examined daily will be diminished.

I shall now enumerate the diseases that are liable to be mistaken for malarial fever. An elaborate description of the differential diagnosis based upon clinical observation is superfluous, since a simple examination of the blood suffices to establish the diagnosis.

The diseases have first to be considered which are characterized by enlargement of the spleen and intermittent fever, such as tuberculosis, sepsis, and endocarditis; and diseases the clinical features

of which resemble those of tropical fever, such as typhoid, yellow fever, and meningitis. Heat-stroke also is liable to be mistaken for commencing malarial fever. Examination of the blood will in all cases, either immediately or at least within twenty-four hours, elucidate the nature of the disease. Further, in all cases of rise of temperature following a wetting, injury, operation, or emotional disturbance in persons who have previously suffered from malaria, we must think of the possibility of malaria, and examine the blood, even when the course of the fever is quite atypical.

The great importance of examination of the blood and the necessity of undertaking it is shown by the following examples, which I have already published in the *Archiv. f. Schiffs. und Trop. Hyg.*

Brandt reports the following case[9]—'Previous history: Sixteen weeks before admission the patient fell on to the deck from a height of 40 feet, was unconscious for several days after the fall, and was confined to bed throughout the voyage. Had previously suffered from malaria. On admission, temperature subfebrile. Aspect that of an imbecile; memory completely lost; speech hesitating; no disturbances of motor or sensory systems; incontinence of urine and fæces. A star-shaped, non-adherent scar on the head; moderate enlargement of spleen. Diagnosis: Pressure on the brain by a depressed fragment of the skull. Trephining to be undertaken. Before operating, however, Brandt examined the blood, and found malarial parasites. Quinine was administered, and four weeks later the patient, whose mental state had become perfectly normal, was discharged cured.'

Bein writes as follows in the *Charité Annalen*: 'Among the cases in which I examined the blood to check the diagnosis, there were three in which the most experienced clinical observer and investigator would have found it difficult in forming his diagnosis to exclude intermittent fever with confidence. More especially a case of pronounced cachexia with enlargement of the spleen and intermittent fever, in the absence of any other sign of organic disease, must have been regarded as malarial in nature. In numerous specimens of the blood, however, plasmodia were never discovered. In two other similar cases, the results of examination of the blood were likewise negative. Further clinical observation and post-mortem examination in these cases showed that we had been justified in excluding malaria from the diagnosis in consequence of the absence of plasmodia from the blood. The autopsy showed the first case to be extensive tuberculosis of the retroperitoneal glands; further observation showed the second case to be carcinoma of the stomach with metastases in the neighbouring organs, and especially in the spleen; while the third case proved to be pulmonary tuberculosis with emphysema and chronic enlargement of the spleen. . . .

'In contrast with these cases is that previously mentioned, in which the diagnosis of malaria was at first very doubtful, and we at first suspected the existence of suppurative processes in the internal organs. The discovery of plasmodia at the very first examination of the blood, however, at once determined the diagnosis.'

VII. PROGNOSIS.

The prognosis is influenced by three considerations.

1. By the nature of the fever—that is, whether the illness is due to infection with the large or the small species of parasites.

2. By the method of treatment.

3. By the environment of the patient.

Fevers due to infection with parasites of the large species are far less dangerous than the tropical fevers due to infection with the small species. In the first place, the large parasites have a far less potent influence on the human organism, so that in uncomplicated tertian and quartan fevers alarming symptoms never occur; and, in the second place, the individual paroxysm of fever lasts, in a case of benign tertian or quartan, one-third only of the time, on the average, occupied by a paroxysm of tropical fever.

If, however, the paroxysms of fever recur throughout a considerable period, if treatment is lacking or inadequate, or if the environment of the patient is an unfavourable one, even a tertian or quartan fever may eventually lead to the appearance of cachexia, or directly threaten life. Further, blackwater fever may ensue. In obstinate relapsing cases of intermittent (benign tertian and quartan) fever the spleen may attain an enormous size, extending downwards into the left iliac fossa, and causing dyspnœa from compression of the lungs consequent on pressure on the diaphragm.

Even when the individual paroxysms of intermittent fever have been cured by quinine, and the patient has been placed amid favourable surroundings, he will remain liable to relapses for a considerable period of time, unless he submits to the prolonged administration of quinine at definite intervals. Earlier in the book I mentioned that quartan fever was characterized by an especial obstinacy in the matter of relapses. Tertian fever, especially that met with in German South-West Africa, exhibits a similar tendency.

In the case of tropical fever the conditions are almost exactly reversed. What here threatens the life of the patient is, as a rule, the actual paroxysm. It may happen that even the very first paroxysm is ushered in with the most threatening symptoms, and that the patient is brought to hospital in a somnolent condition. In such cases the prognosis must always be regarded as very uncertain. But in tropical fever, in addition to somnolence and coma, other symptoms may appear which greatly increase the gravity of the prognosis.

Sudden and profound collapse may occur, or with equal suddenness an algid condition may come on, in which the patient suffering from tropical fever resembles a cholera patient *in extremis*.

On the other hand, when the first attack of tropical fever is energetically treated, relapses are less common than in the case of intermittent (benign tertian and quartan) fevers. Cases of tropical fever do, indeed, occasionally occur in which relapse follows relapse, but this is exceptional. When these frequent relapses occur the prognosis is naturally unfavourable, since anæmia, dilatation of the right side of the heart, and gradual enlargement of the spleen culminate in cachexia.

Examination of the blood gives information of decisive value in forming a prognosis, and there is yet another respect in which examination of the blood aids prognosis if we use the term in its wider significance (foreknowledge).

Since, as we have seen, the course of the fever has a quite definite relationship to the development of the parasite, we are able by examining the blood not only to determine the nature of the parasite, and thus to form a prognosis in the ordinary sense of the term; but, further, from the stage of development in which we find the parasite to be, we can predict the time of onset of the next paroxysm. If, for instance, we find tertian parasites, and, let us suppose, only full-grown parasites and fission-forms, we can predict the next paroxysm to be due immediately. Should we find half-grown forms only, we expect the paroxysm in twenty-four hours; whilst, should we find small tertian rings only, we know that the next paroxysm is not due for from thirty-six to forty hours. The prognosis by means of examination of the blood in cases of quartan fever is carried out on similar lines.

Such prognoses, however, can be made with certainty only in cases of recent infection; in chronic fevers a certain degree of irregularity in the development of the parasites always becomes apparent. In such cases it may happen that, for example, in the evening we find full-grown and almost full-grown forms, and therefore predict that the next paroxysm will occur on the following morning. Yet, notwithstanding the fact that no quinine has been administered, the paroxysm fails altogether to appear. In the morning, indeed, we find that the parasites have almost entirely disappeared from the blood, fission not having taken place, probably because, in cases of chronic intermittent fever, a certain degree of immunity has always already been established.

In cases of tropical fever such prognoses as those just described cannot be made, because the paroxysms run a less definite course, and do not succeed one another so regularly as the paroxysms of intermittent fever dependent on infection with parasites of the large species. We are, indeed, able to assert that the end of the paroxysm is approaching as soon as we find large tropical rings; but we are not able to determine in how many hours the new paroxysm will begin.

VIII. THERAPEUTICS.

In quinine we undoubtedly possess a specific against malaria, but we must not therefore be misled into using our specific in an uncritical manner. We must, on the contrary, inquire, and inquire with great precision, when and how it may be administered with the greatest possible advantage.

As long as two hundred years ago—about sixty years, that is to say, after the discovery of the antipyretic action of cinchona bark—the English fleet-surgeon Cockburn reported (1697) that quinine was effectual only when administered during the apyrexial period. In the latter half of the eighteenth century the English naval surgeons Lind and Blane called attention to this point once more, and gave most masterly directions as to the treatment, both of the indigenous and the tropical varieties of malarial fever. In course of time, however, some uncertainty arose in regard to the administration of quinine, because the drug came to be regarded as untrustworthy in the treatment of tropical fever. This apparent inefficacy of quinine was to be explained by good reasons. For, first, the practice had arisen of giving quinine not only during the apyrexial period, but also during the height of the fever; secondly, no attention was paid to the question whether the quinine that had been administered was really absorbed into the circulation; and, thirdly, quinine was employed in the treatment of fevers which were not malarial in nature. Erroneous practices of this character have continued to cause confusion down to the present day.

After the discovery of the malarial parasites, and more especially after the intimate study of their life-history by Italian observers, the conclusion was drawn that quinine was especially deleterious to the young forms of the parasites, and that the drug should therefore be present in the blood simultaneously with these young forms. Golgi therefore advised, in accordance with the old empirical rule, that in

intermittent fevers quinine should be administered four or five hours before the expected paroxysm. This method of administering quinine is essentially sound. It is, however, far more probable that quinine does not attack the very young forms of the malarial parasites, but, on the contrary, that the drug is especially inimical to the oldest parasites, the fission-forms (sporulation-forms). Quinine does not actually kill these, but merely, according to Koch's view, checks fission (sporulation). The essential accuracy of this hypothesis appears to be established when we remember that the onset of the fever is associated with fission (sporulation), and is doubtless directly due to this process. If, in consequence of an accurately timed and sufficiently large dose of quinine, the impending paroxysm of fever fails to occur, we infer that fission (sporulation) of the parasites has not taken place.*

Now, indeed, it was definitely established that in the manner above described intermittent fevers could be effectively treated; but hitherto no scientific basis had been found for the old empirical rule that in tropical fever quinine must be administered during the apyrexial period, and for this reason even at the present time quinine is by many physicians improperly administered in tropical fever during the height of the fever. But here also we have been helped by R. Koch. By exact observation of paroxysms of fever uninfluenced by the administration of quinine and their relation to the various forms of the tropical parasites, he ascertained that these parasites complete their development in the peripheral blood in the form of the large tropical rings shortly after the paroxysm of fever—at the beginning, that is, of the apyrexial period.† Since, however, fission (sporulation) of the tropical parasites takes place in the capillaries of the internal organs, and since from the appearance of the large tropical rings to the completion of fission (sporulation) from six to eight hours or more may elapse, it is necessary, if we wish to prevent fission from taking place, and thus to prevent the occurrence of the new paroxysm of fever, to administer quinine, in accordance with the

* Whereas we can only infer from the reasons just given that quinine is effective in the manner above described, we can, in the case of methylene-blue, directly observe this mode of action. As Temperature-Chart II. shows, methylene-blue does not kill off the quartan parasites, but merely prevents the occurrence of sporulation. Notwithstanding the administration of methylene-blue, we may continue to find parasites in the blood for four days; but the drug prevents fission of the parasites, and therefore we find no fission-forms, and no further attacks of fever occur.

† This fact enables us, in the case of an incomplete remission, to determine whether the paroxysm of fever is or is not finished.

old empirical rule, at the beginning of the apyrexial period, and not during the height of the fever, for quinine given at this latter time has already for the most part been eliminated from the blood at the time when fission (sporulation) of the parasites takes place.

In cases in which, notwithstanding the most accurate observations of the temperature, no apyrexia can be observed, but merely a remission, we may administer quinine with expectation of good effect during this remission (which may be exceedingly slight,* as is seen, for instance, in Temperature-Chart V., Temperature-Chart VII. on the third day, and Temperature-Chart VIII. on the second day), as soon as the large tropical rings make their appearance. If, however, during such a partial remission, we find small or medium-sized rings, we know at once that the attack is not yet over, and that we are dealing only with the pseudo-critical depression; we know, further, that for the present fission (sporulation) of the parasites cannot be expected to occur, and therefore that the time is not yet ripe for the administration of quinine. It has more than once happened that the pseudo-critical depression usually exhibited by the curve of tropical fever has been regarded as the beginning of the true crisis, that quinine has then been administered without effect, and the conclusion drawn that quinine is also ineffectual in the remissions of tropical fever.

We come now to the question of the dosage of quinine.

In recent intermittent fever (benign tertian and quartan) 1 gramme (15 grains) of quinine given four or five hours before the anticipated paroxysm usually suffices to suppress the fever. The next paroxysm, indeed, often occurs; but it is weaker, and more especially are the subjective symptoms less severe. For instance, in the paroxysm occurring after quinine has been given, the rigor is almost always absent. The case is altered when we have to do with intermittent fevers of longer standing. The paroxysm of fever, in anticipation of which quinine has been given, here usually occurs (see Temperature-Chart IX.), but somewhat mitigated in its severity. We must therefore continue on successive days at the same hour to administer 1 gramme (15 grains) of quinine until the fever is suppressed.

We must not, however, believe that with 1 gramme (15 grains) of quinine, even when in consequence of this dose the anticipated paroxysm has failed to appear, an attack of benign intermittent fever can really be cured. This would be a most dangerous error. Even in cases in

* It must not be inferred from this that I recommend the administration of quinine during the height of the fever.

which after 1 gramme (15 grains) of quinine has been given the fever has to all appearance been promptly suppressed, we must on the six ensuing days repeat the dose of quinine at the same hour. Then only we may assume that the fever has for a short time been really overcome. If, however, slight rises of temperature which cannot be regarded as due to any complications continue to occur, we must, of course, continue to administer quinine until the fever has entirely ceased.

Nor does it often happen that tropical fever is cut short by a single dose of 1 gramme (15 grains) of quinine (*cf.* Temperature-Charts V., VI., and VIII.), even if this dose is administered at the right time, when the large tropical rings appear in the blood.* For in this kind of fever fission (sporulation) occupies a longer period, and for this reason quinine must be present in the blood for a longer time if the fission of all the parasites is to be prevented. Hence the first dose of quinine must be followed in three or four hours by a second dose of 1 gramme (15 grains) or of half the amount, and after a similar interval a third dose must be administered (*cf.* Temperature-Chart XIV.). The second and third doses may be reduced to ½ gramme (7½ grains) each, because when they are given some quinine will still remain in the blood, a portion of the previous dose.

If 1 gramme (15 grains) only of quinine has been given, and the next paroxysm ensues as usual, further administration of quinine in the manner above described can only be undertaken with effect when this paroxysm has run its course. In cases of tropical fever this is disagreeable to the patient, for the paroxysm that follows the first dose of quinine may last from twenty-four to thirty-six hours. But we must not be misled into beginning the second administration of quinine during the height of the fever in the hope of cutting short the paroxysm, for this will not have the desired effect. On the contrary, after the administration of quinine during the height of the fever, we see no result beyond an increase of the subjective discomforts of the patient. The course of the fever itself remains quite unaffected.

We may summarize what has just been written into the following general directions for the administration of quinine:

1. In intermittent (benign) fevers we must give 1 gramme (15 grains)

* Even though by a single dose of quinine administered at the right time the impending paroxysm of fever is not usually prevented, still, the result of the administration is usually that a complete intermission follows this paroxysm, although after the first paroxysm there had been a remission merely of the fever (*cf.* Temperature-Charts V., VI., and VIII.).

of quinine* four or five hours before the impending paroxysm, and repeat this dose at the same hour on six successive days.

2. In tropical fevers we must give 1 gramme (15 grains) during the decline of the fever, at the time when the large tropical rings make their appearance in the blood, and this dose must be repeated in four hours; or ½ gramme (7½ grains) may be given in four hours, and the same dose repeated four hours later. At the same hour on six successive days 1 gramme (15 grains) of quinine must be administered.

There remain, however, certain special circumstances to be taken into consideration in connexion with the administration of quinine, for in certain fevers that are truly malarial quinine may be administered at the proper time, and may yet at first appear to have no effect.

This may depend on complications which diminish or altogether destroy the absorptive capacity of the stomach. We are not here necessarily concerned with disease of the stomach itself, for the deficient power of absorption may depend on some other disease. For instance, I refer the failure of the quinine, administered at the proper time, to control the fever that is manifested in Temperature-Chart XIX. to the fact that the patient was simultaneously affected with dysentery.

But we have also to remember that quinine is soluble in sufficient quantities only in acid fluids.

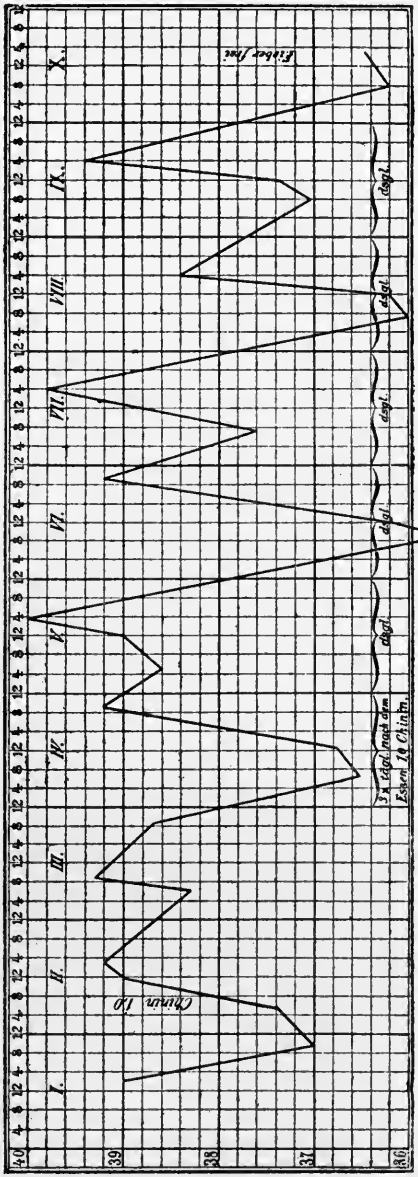
The solution and absorption of the necessary quantity of quinine can therefore take place in the stomach only. If the stomach is distended and its contents are rapidly passed on into the alkaline intestine, a small proportion only of the quinine administered will be dissolved and absorbed. A striking example of this is seen in Temperature-Chart XXI. Here first of all on the second day 1 gramme (15 grains) of quinine was administered, but we see that this was simultaneous with the ascent of the fever-curve, and was therefore too late, since the fission of the parasites was already in progress. From the fourth day onward, 1 gramme (15 grains) of quinine was administered thrice daily without any effect, *because the quinine was always given after meals.* Formerly quinine was often administered in this manner in the hope of diminishing or altogether avoiding the disturbing influence of the drug on the mucous membrane of the stomach.

* For young children the dose of quinine given by mouth is 1 decigramme (1½ grains) for each year of their age (R. Koch).

As this Temperature-Chart XXI. dates from the year 1897, it is clear that it has been drawn up *sine ira et studio*.

It is well, therefore, to give quinine early in the morning on an

TEMPERATURE-CHART XXI.



Fifteen grains of quinine thrice daily after food. The same. The same. The same. The same. The same. The same.

FIRST INFECTION WITH TROPICAL FEVER (CAMEROONS).

empty stomach, and in cases of gastro-intestinal catarrh, or when the stomach has an alkaline reaction, to inject quinine hypodermically, as recommended by R. Koch.

Both the form of the drug used* and the method of administration are of the greatest importance. Quinine is usually given in the form of powder, wrapped up in wafers. This is a satisfactory method. But in remote stations in the colonies and on board small gunboats in out-of-the-way parts of the world, such as the South Seas, the supply of wafers is often exhausted without possibility of renewal. The quinine, the intense bitterness of which is dreaded by most patients, is then given in cigarette-papers. I adopted this method myself in Zanzibar twelve years ago, and was then extremely pleased to find how well I 'bore' the drug. On each of two successive days I had taken for prophylactic purposes 1 gramme (15 grains) of quinine without a trace of tinnitus aurium or gastric discomfort ensuing. So much the more was I surprised a year later in Germany, when, believing I was suffering from larval malaria, I again took quinine, this time in a wafer. After a single dose of 1 gramme (15 grains) at bedtime, next morning I suffered from severe tinnitus aurium and a flickering sensation in the eyes. Evidently on the former occasion, when the quinine had been wrapped in cigarette-paper, the drug had not been absorbed, whereas on the latter occasion it was apparently all absorbed.

Tabloids of quinine are as useless as quinine wrapped in cigarette-paper, for they are passed undissolved in the stools. This also occurs with old quinine pills. R. Koch observed that in acidulated water they remained undissolved for days. R. Koch also rejects the use of quinine in gelatin capsules, for these often contain only half the alleged amount.

Since, then, these methods of administration are either inapplicable (as from lack of wafers) or are altogether valueless, R. Koch has returned to the primitive method, the use of a solution of quinine, which he recommends especially for administering the drug to large numbers of persons. As a corrective, a piece of sugar is given after each dose. In dealing with individual patients we always endeavour to use wafers. If in this method a little quinine is by inadvertence spilled on the tongue, a piece of apple-rind is an excellent corrective.

If the quinine is administered by subcutaneous injection, or by intramuscular injection after the manner in which injections of mercury are given, the most elaborate antiseptic precautions must be taken.† Half a gramme (7½ grains) is a sufficient dose. The

* On German men-of-war for several years past the hydrochlorate of quinine has been used exclusively, and has been administered in wafers.

† In Zanzibar tetanus has been observed after injections of quinine.

quinine is dissolved, the solution is sterilized, and is hermetically sealed in small glass tubes, each containing ½ gramme (7½ grains) of bim. carbam. of quinine. These tubes are prepared by Kade's Oranien dispensary. Subcutaneous injections of quinine are very painful, and are followed by disagreeable indurations at the seat of injection, but they are very effective, and are not followed by the disagreeable subjective symptoms so often seen when large doses of quinine are given by mouth.

Why it is that these subjective symptoms are absent when quinine is administered by subcutaneous injection, although the quinine is absorbed, as is manifested by its striking effect on the malarial parasites, has not yet been explained.

Nor do we yet understand why it is that quinine is dissolved by the alkaline tissue juices and by the alkaline blood, but not by the alkaline intestinal secretion.

How far quinine is absorbed when administered by enema cannot yet be determined with certainty, since exact observations on this point are still lacking.

Having now considered the time of administration, the dosage, and the method of administration of quinine, we must turn to the important question how long the drug must be given in order to prevent any return of the fever.

We have already seen that a single dose of 1 gramme (15 grains) of quinine seldom suffices to cut short an attack of malarial fever. Still less does such a quantity suffice to prevent relapse; nor even is it effective when given several days in succession, and then again at longer intervals. In this respect Temperature-Chart XV. is very instructive.

In this case the fever was apparently checked by a single dose of 1 gramme (15 grains) of quinine, and on the two following days the dose was repeated, so that the patient had taken 3 grammes (45 grains) in all. Subsequently every tenth day 1 gramme (15 grains) of quinine was administered. In spite of this, four weeks later he had a relapse, lasting three days. During this relapse he was twice given 1 gramme (15 grains) of quinine subcutaneously. Nine days later he had another relapse, also of three days' duration. On three successive days, beginning with the second day of this relapse, 1 gramme (15 grains) of quinine was administered. Fourteen days later came yet another relapse, which, after the administration of 1 gramme (15 grains) of quinine, was complicated by an attack of blackwater-fever. During the ensuing five weeks no quinine was given, although

in this period the patient suffered from several attacks of fever. Then an experimental dose of ¼ gramme (4 grains) of quinine was given, which resulted in a second attack of blackwater-fever, in consequence of which the patient died during the voyage home.

We learn that 1 gramme (15 grains) of quinine every tenth day is insufficient to prevent the occurrence of relapses.

It is necessary, in order to control the fever completely and permanently, to administer quinine on two successive days, in doses of 1 gramme (15 grains) each day, at least every tenth and eleventh day. This matter will be more fully discussed in the section on prophylaxis.

I shall now consider briefly other points in the treatment of malarial fever.

To relieve the distressing headache, ½ gramme (7½ grains) of antipyrin or ¼ gramme (4 grains) of phenacetin will be found very effective. Obstinate insomnia is best treated with chloral-hydrate, if the stomach will tolerate the drug. Injections of morphine are to be reserved till other means have failed to procure sleep. Vomiting, also a most distressing symptom, can often be relieved by morphine in doses of 0·015 gramme (⅓ to ¼ grain). In especially obstinate cases, when morphine fails to relieve the vomiting, chloroform (2 or 3 drops in water) is of service.

A warm bath will under certain circumstances prevent an impending relapse. Naturally, however, in such a case, we must not fail to treat the patient with quinine.

The only drug which, besides quinine, has a direct influence on the malarial parasites is methylene-blue. Its influence is, however, much weaker than that of quinine. The only preparation that should be used is methylene-blue medicinale purum Höchst. It is given in doses of 0·2 gramme (3 grains) five times a day, in gelatin capsules, and its administration is continued for eight days. It is employed in cases in which blackwater-fever has followed the administration of quinine. The therapeutic use of methylene-blue has already been discussed in fuller detail in our account of the treatment of blackwater-fever. Temperature-Chart II. shows the mode of action of methylene-blue (*cf.* note to p. 63).

IX. PROPHYLAXIS.

Prophylaxis may be considered under two headings—individual prophylaxis and general prophylaxis.

(a) Individual Prophylaxis.

Individual prophylaxis—that is to say, the attempt to ward off attacks of fever by the prophylactic use of quinine, was, I believe, first undertaken by Count Bonneval during the Siege of Belgrade in the year 1717. As Kramer reported in 1737, this attempt of Bonneval's was successful; he himself and his servant, who also took quinine, remained free from fever. Unfortunately, we are given no information regarding the dose used. The English naval surgeon Lind, towards the end of the eighteenth century, treated this question more in detail. He proposed that those members of a ship's complement who were compelled to pass the night on shore should take every day 0·7 gramme (10 grains) of quinine.* At a later date opinions varied as to the magnitude of the dose of quinine required for prophylactic purposes. English physicians, in their so-called 'quinine-schnaps,' gave only 0·3 gramme (5 grains) of quinine, and inasmuch as this dose is ineffective, this practice cast discredit on the prophylactic use of quinine. Quite recently this question has aroused great and renewed interest. A. Plehn, especially, has been investigating the subject, but has not as yet attained any very striking results, as will be seen in the appended report by Schroeder. R. Koch also has studied this subject, which is of such great importance from a hygienic standpoint, and, as a result of numerous experiments, has come to the conclusion that isolated large doses of quinine, even if taken at comparatively short intervals, are not sufficient permanently to ward off fever. He ascertained that a large dose must be given on each of two successive days. He recommends the administration of 1 gramme (15 grains) of quinine on the morning of every tenth and eleventh day, in order to prevent the occurrence of fever. If, notwithstanding this, fever sets in, either the dose of quinine should be increased to 1½ grammes (23 grains), or else the days of administration should be changed to the ninth and tenth. Quinine may be administered in this manner for a long period without disturbing the stomach.

* The drug used by Lind was cinchona bark, not quinine. It is on the assumption that the bark contains 5 parts per cent. of quinine that the above-mentioned daily dose of quinine has been calculated.

In 1898 Staff-Surgeon Schroeder, of the Imperial German Navy, stationed off the coast of the Cameroons on board S.M.S. *Habicht*, made some observations on the prophylactic use of quinine, and reported on the subject as follows[10]: 'Prophylaxis of malaria, in the sense that quinine or arsenic was universally administered with a view to preventing the disease, was not attempted. But all those who had once suffered from malaria were subjected by A. Plehn to treatment with quinine, ½ gramme (7½ grains) every five days. As regards the prevention of relapses, this measure, as carried out on board the ship, appeared to me to have no effect.

'According to Ruge, that the blood may contain quinine in quantity sufficient to be effective, the minimum dose required for an adult is 1 gramme. Latterly, in accordance with this view, I have increased the dose of quinine administered prophylactically, giving on the seventh day 1½ grammes (23 grains), and on the eighth day 1 gramme (15 grains); on the fifteenth day a dose of 1½ grammes (23 grains) is once more administered, and on the sixteenth day a dose of 1 gramme (15 grains); and the administration of the drug in these doses and at such intervals is continued throughout a period of six weeks.

'Thus the same quantity of quinine is given as if we had to do with a patient actually suffering from fever. These doses of quinine have hitherto always been well borne, and, further, relapses have ceased to occur.'

This method of administering quinine differs from the methods hitherto employed in this important respect, that it is founded upon the observed fact that two successive doses of quinine have a much more powerful effect than one isolated dose.

This prophylactic use of quinine is the only means by which in malarial regions fever can be permanently prevented, and to persons who have suffered from fever — whether benign, intermittent, or tropical fever is indifferent—we must also give 1 gramme (15 grains) of quinine every tenth and eleventh day if we wish to prevent the occurrence of relapses. Even when malarial patients remove to regions in which they are no longer exposed to fresh infections, as, for instance, when they return to their homes in the North, the treatment with quinine, above described, should be continued for not less than three months if they wish to be permanently cured of malaria.

Besides this treatment with quinine, all other measures of personal prophylaxis have a secondary or transient importance only, inasmuch

as, in respect of certainty of action, they cannot for a moment be compared with the prophylactic use of quinine.

For example, the rubbing into the skin of certain ethereal oils, such as oil of cloves, has been most widely recommended to prevent the bites of mosquitoes, and thus to prevent malarial infection. In my experience this measure is effective only so long as the smell of the oil of cloves remains distinctly perceptible, which is usually about half an hour. After this the mosquitoes began to bite once more. I do not know how long a skin weakened by profuse secretion of sweat would bear without injury the repeated application of oil of cloves, but certainly not very long. Even in our own climate, after repeated application of oil of cloves to the skin, a certain discomfort is experienced. To what degree the frequent application of ethereal oils may tend to injure the kidneys has not yet been worked out.*

The use of Kummerfeld's lotion causes no irritation of the skin, and gives, according to my own observation, more effective protection from mosquito-bites. This lotion contains sulphur in suspension, and as the fluid evaporates the sulphur is left in a thin layer on the skin. I was led to employ this lotion because of the statement frequently made by Italian physicians that the labourers in sulphur-mines do not suffer from malaria.

Another protective agent, and a most useful one, is the mosquito-net. This must never be neglected. Indeed, in tropical countries undisturbed sleep cannot be procured without a mosquito-net, and undisturbed sleep is far more essential to Europeans in the tropics than it is at home. Another measure that has been recommended to travellers is to keep up a large fire throughout the night in front of the opening of the tent. This is also a useful practice, for the mosquitoes are drawn into the upward current of air caused by the fire, and are consumed in the flames.

Quite recently it has been recommended that the windows and doorways of the houses of Europeans in the tropics should be covered with mosquito-proof wire gauze, that in this way the inhabitants may be protected from the entry of the bearers of infection. This measure is based on the results of the experiment made by Sambon, Low, and Rees in the neighbourhood of Ostia. They lived in a malarial region

* Translators' Note.—Of the ethereal oils the most effective, in our experience, in preventing mosquito bites is citronella oil. It is also one of the cheapest of these bodies. We have not seen any ill-effect from long-continued use, and a single application remains effective for an hour or more. But its powerful odour is very disagreeable to many nostrils.

in which most people suffered from chronic malarial cachexia, in a house the windows and doors of which were protected in the manner just described, so that no mosquitoes could enter. They slept under mosquito-nets. They left the house only between the hours of sunrise and sunset, and took every possible precaution to avoid mosquito-bites. Living in this manner for two months during the malarial season they remained in good health.

The accuracy of this experiment is not open to doubt. But, in the first place, control experiments were not made; and, in the second place, it is quite impossible for most Europeans in tropical and subtropical countries to live under such conditions as those described—think, for example, of military enterprises, excavations, and plantation-works. In various occupations people are absolutely compelled to expose themselves to the night air at the time when the anopheles chiefly swarm. And if people living under the conditions described are once infected—and an opportunity for infection will occur sooner or later—the mosquito-proof house is of no further use. Further, a tropical house cannot be rendered permanently mosquito-proof. Everyone who has lived in the tropics knows that in the evening the servant must be sent to kill the mosquitoes that have found their way into the interior of the net over the bed. If, however, mosquitoes penetrate into the interior of an apparently perfect mosquito-net, we may be certain that they will get into a house, the doors and windows of which must occasionally be opened, even if the doors close automatically. We must also bear in mind that the provision of wire-netting for many tropical houses, built after the present fashion, can be effected only at great expense, and that after all our pains, the little *Anopheles funestus*, which, according to Daniels, is found throughout Central Africa, finds its way even by day into houses and through mosquito-netting. And tropical houses as at present constructed become unendurably hot when all openings are occupied with wire-netting (Daniels).

In a house thus enclosed by wire-netting, mosquitoes, once inside, are far more likely to remain permanently than in an unprotected house. But they cannot, as in a mosquito-net, be readily found and destroyed. Hence, if we have a man infected with malaria in such a house, and the two or three anopheles it may contain are infected by him, the house at once becomes a focus of infection.

Even if the mosquito-proof house fully effected its purpose, and its inhabitants remained in good health, we should still have by this means protected only a small fraction of those living in the district.

The anopheles would still have opportunities to infect afresh, or themselves to receive infection from, that far larger portion of the population which cannot spend the whole night in mosquito-proof houses, and whose occupation brings them into contact with mosquitoes in the daytime also, such as harvest labourers and coolies on coffee plantations.

One who cannot take quinine at all, or bears the drug badly, and whose circumstances are such that he can spend the whole night indoors, is *perhaps* in a position to ward off malaria permanently by the use of mosquito-nets. I say *perhaps* because the anopheles of different regions have different habits. Ziemann, for example, reports that in the Cameroons anopheles bite vigorously during the daytime also.

Further, the observation that in the tropics it is the children of the indigens that are chiefly the hosts of the malarial parasites, and more especially of the gametes, has led to the recommendation that the Europeans should live in places removed as far as possible from native habitations. This recommendation is supported by the fact that the members of the English malaria expedition to Lagos found that the anopheles in the native huts were not only infected during the fever season in the proportion of 50 per cent., but were infected even during the dry season in the proportion of 5 to 10 per cent. This observation is especially noteworthy because in the dry season anopheles in large numbers are to be found only in the huts of natives. Even during the fever season in the houses of Europeans anopheles are found in small numbers only.

The above recommendation is in itself an excellent one, but the following considerations stand in the way of its being carried out. We have seen that the anopheles fly at least 1,500 metres (1,640 yards), but often further, and by winds may be carried much further. Therefore the houses of the Europeans would have to be built at least two kilometres (a mile and a quarter) distant from the native huts, in order to obtain protection with any degree of certainty. This measure is, however, impracticable.

I must, therefore, reiterate that the only rational and everywhere applicable method of individual prophylaxis is that advocated by R. Koch and Schröder.

(b) General Prophylaxis.

It has been believed that general prophylaxis could best be achieved by a generalization of individual prophylaxis by the use of quinine. This generalization, however, is by itself insufficient to give us mastery

over malaria. Therefore I regard as purposeless the introduction in our colonies of compulsory quinine-prophylaxis, even apart from the fact that it would be quite impossible to insure its complete enforcement.

As further means of general prophylaxis measures have been recommended that will bring about the destruction of mosquitoes. Very various proposals have been made. Some measures are directed against the winged insects that have found their way into habitations; others are directed against the larval forms. This last means only, under quite peculiarly favourable local conditions, and in a limited area, may be expected to have a temporarily successful result. The best way is to pour petroleum into the puddles containing the larvæ, or else to throw lime into them. We can well understand that if this is done regularly and for a considerable period of time (as by Ross during three months on every alternate day), in the immediate neighbourhood of inhabited houses or villages offering facilities for carrying out the necessary measures, a certain result may be obtained. But a short interruption to the operation of the necessary measures will be followed by a loss of the results previously gained.* It may be added that persons who have benefited from these measures at some particular place cannot always stay in this place, but have to visit others, in which latter they will lose the advantage of the larva-destroying operations. When we take further into consideration the fact that by air-currents mosquitoes may be conveyed to a distance of five kilometres (three miles) from their breeding-grounds (Wenzel),† we see that it would be necessary to destroy the larvæ of mosquitoes in an area with a radius of at least five kilometres (three miles). Anyone who has ever visited the tropics and has seen tropical marsh-vegetation and the incredible rapidity with which it grows again over cleared areas, will at the outset reject the idea of a general destruction of mosquito larvæ; and even if he should venture on the undertaking in some especially suitable area, he is not likely to try a second time. Moreover, as already pointed out, such measures are only of use for the quite stationary portion of the population. Who will believe that by such means as these we can improve the hygienic conditions of

* Stephens and Christophers report that eight days after they had ceased to pour petroleum into the little puddles and gutters in Freetown that had contained larvæ of anopheles, the larvæ, which during the operation had disappeared, were again to be found.

† Ziemann states that in the Cameroons he never found the breeding-places of anopheles at a greater distance from human habitations than 1,500 metres (1,640 yards).

a troop of men marching and fighting by turns, or of men engaged in a survey of the coast-line?

Since, therefore, neither a generalization of individual prophylaxis nor any of the above-mentioned measures which have been proposed for the purpose of general prophylaxis, can be regarded as likely to be effective in freeing a country from malaria, R. Koch has introduced a new method.

This method of R. Koch, by means of which in a few months he freed Stephansort, one of the many malarial districts of New Guinea, from malaria, without disturbing the people in their habits and occupations, aims at the actual extermination of malaria.

R. Koch started with the assumption that malaria should be combated in the same manner as plague or cholera—that is, by searching especially for the slight cases which do not come under the notice of the physician, and therefore contribute most to the spread of the disease, and by rendering these cases harmless.* This can be done easily enough in the case of malaria by means of quinine. By systematic examination of the blood it was first determined what persons were actually suffering from malaria. All these patients were cured by means of quinine; not only the first infections, but also the relapse cases which form the connecting-link between the annual epidemics of malaria, were cured and rendered harmless.

If we wish, therefore, to be successful in the quinine treatment in a place where it is desired to exterminate malaria, it is necessary not only to combine the method of R. Koch with the improved method of personal malarial prophylaxis (the latter is necessary for all newcomers), but special attention must be paid to the following points:

1. The quinine used must be pure, not, as was found by North[12] in Italy, adulterated by 80 per cent. of starch.

2. The quinine must be administered in a proper form, not in pills, cigarette-paper, or tabloids.

* In this connexion Ziemann[11] reports as follows: 'It may be of practical value to note that recently in Europeans several cases were observed in which the temperature scarcely reached to 37·5° C. (99·5° F.), although the development of the parasites nevertheless took its uniform course. The patient all the time was entirely free from the subjective sensations that accompany fever. In these isolated cases we have always to do with anæmic persons who have already suffered much from malaria. . . . It seems that this condition is found more frequently in negroes. In them the subjective symptoms are far less pronounced than in the white race, and often are altogether absent.

3. It must be administered at the right time, both in respect of the condition of the parasites and in respect of meals; and possible complications must be watched for, which might modify or reduce the power of absorption of the stomach.

4. The quinine must be given in proper doses.

5. The quinine must be given for a sufficiently long time.

APPENDIX

On the advice of Privy Councillor R. Koch, the firm of F. and M. Lautenschläger has constructed a travelling microscope. The case containing the microscope encloses also two little trays, one of which is empty, and can be filled according to the wishes of the owner, while the other contains the following instruments and objects necessary for microscopical study:

1 pair of microscopical scissors.
1 camel-hair brush, 8 centimetres (3 inches).
1 pair of Ehrlich forceps.
2 Kolle's needle-holders.
1 pipette of 1 c.c. (16 minims) capacity.
1 pencil.
5 object-glasses (hollow ground).
10 object-glasses (ordinary).
3 bottles of staining reagents, with screw top (methylene-blue, gentian-violet, and fuchsin).
2 tubes of cedar-oil.
1 tube of vaseline.
1 spirit-lamp.
2 pairs of Cornet's forceps.
100 cover-glasses, 18 millimetres ($\frac{3}{4}$ inch).
1 box of labels.
1 gramme (15 grains) of platinum wire.
1 lancet for inoculation.
Gutta-percha.
Pieces of chamois-leather.
Pieces of linen.

GENERAL EXPLANATIONS TO THE PLATES.

The following plates have been prepared, for the most part, from my own preparations. The photographs have been made by that master of microphotography, Professor Zettnow, and have been reproduced according to the method of the new Photographic Society in Steglitz, near Berlin.

Of the mosquitoes I have given the external appearance only, the object of these figures being to enable the reader to differentiate a culex from an anopheles. The *Culex annulatus* has also found a place in the plates, as it may easily be confounded with *Anopheles maculipennis* (Meigen) by superficial observers. The two species of anopheles from the Cameroons and from Zanzibar respectively are intended to show the striking differences between the tropical and the European species of

anopheles. Since at present the classification of mosquitoes is still a much disputed one, I have not attempted to name the two tropical species of anopheles.

PLATE I.

Figs. 1-34: Forms of the endogenous development of the human malarial parasite.

The Figs. 1-6 represent the three different species of the human malarial parasites, and are magnified 500 diameters. They represent the appearances seen in an ordinary examination (Leitz $\frac{1}{12}$ homog. immers., eyepiece 1).

Figs. 1-4 stained by Romanowsky's method; Figs. 5 and 6 simple methylene-blue staining.

Fig. 1: Tertian parasite, a few hours old. There is some indication of the fine stippling characteristic of the tertian parasite.

Fig. 2: Small tertian ring.

Fig. 3: Half-grown tertian parasite, about twenty-four hours old (large tertian ring). The characteristic stippling fully developed. Infected erythrocyte distinctly enlarged.

Fig. 4: Nearly full-grown quartan parasite. Infected erythrocyte not enlarged (this cannot be recognised in the plate).

Fig. 5: Small, intermediate, and large tropical rings. The infected erythrocytes not enlarged.

Fig. 6: Gametes of the tropical parasites. Three spheres and one crescent. Preparation of bone-marrow. The crescent is torn.

The Figs. 7-48 are all magnified 1,000 diameters, the only exception being Fig. 43, which is magnified 500 diameters. This great magnification is necessary to show the details of the specimens.

Figs. 7-12: Development of the tertian parasites.

Figs. 13-18: Development of the quartan parasite.

Figs. 19-24: Development of the tropical parasite.

All stained with methylene-blue.

Fig. 7: Small tertian ring.

Fig. 8: First growth of the ring. Commencing enlargement of the infected erythrocyte.

Fig. 9: Half-grown tertian parasite, so-called amœboid form.

Fig. 10: Tertian parasite at the stage of three-fourths of its full development. Infected erythrocyte enlarged to double its original size.

Fig. 11: Dissolved fission-form (seventeen young parasites). The black spot in the middle is the aggregated pigment.

Fig. 12: Free gamete (sphere), the pigment scattered over the whole parasite.

Fig. 13: Quartan ring.

Fig. 14: The ring is drawn out lengthwise, assuming the ribbon form.

Fig. 15: Narrow quartan ribbon.

Fig. 16: Broad quartan ribbon.

Fig. 17: Fission-form (eight young parasites). The black dot in the middle is the aggregated pigment.

PLATE I.

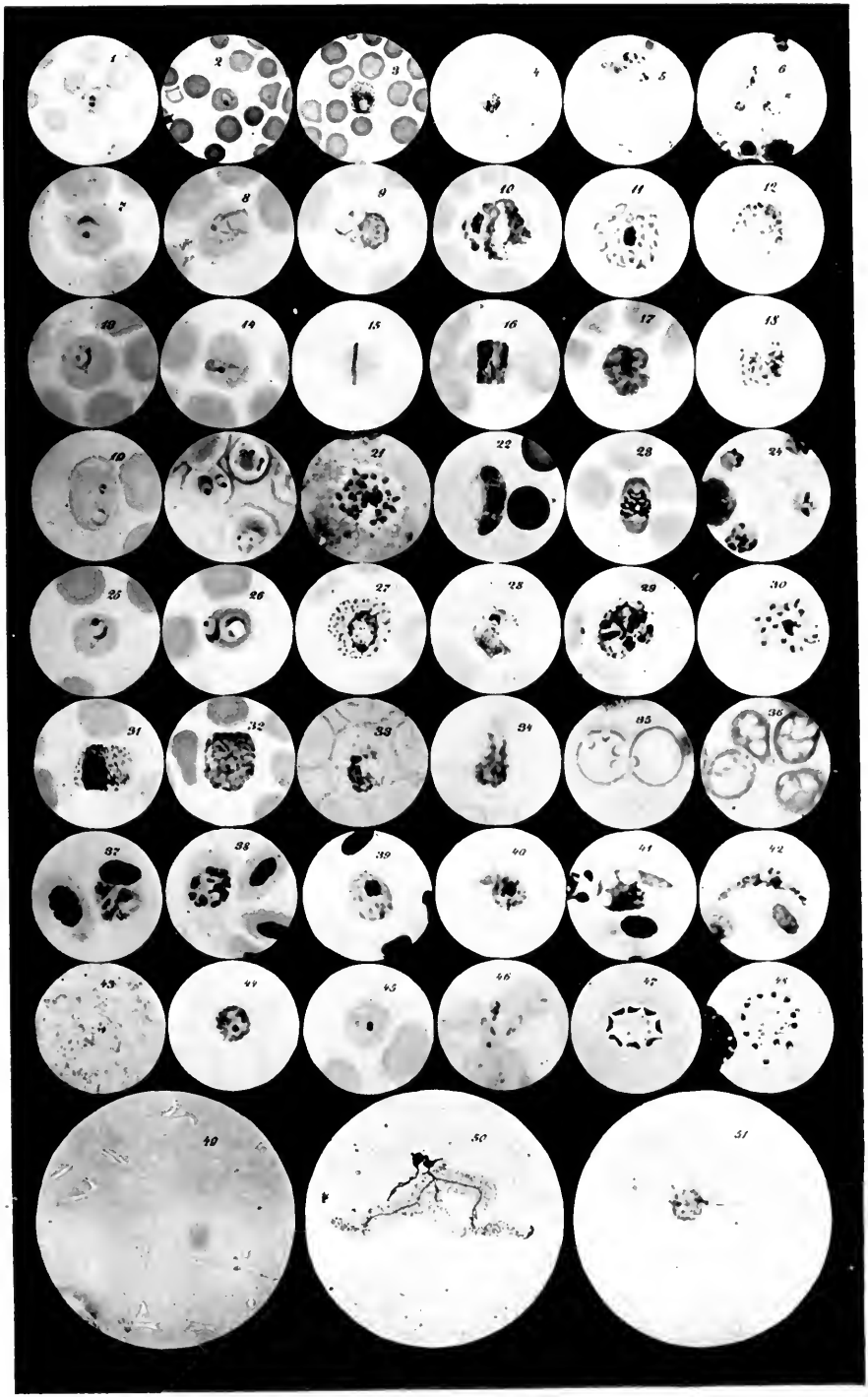

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Fig. 18: Free gamete (sphere). Pigment scattered through the whole parasite. This is under certain circumstances not to be differentiated from a tertian gamete (*cf.* note on p. 11).

Fig. 19: Small and medium-sized tropical ring.

Fig. 20: Intermediate and large tropical rings.

Fig. 21: Fission-form of the tropical parasite (twenty-two young parasites). Preparation from bone-marrow. The large black spot in the middle is the aggregated pigment.

Fig. 22: Crescent. The remainder of the erythrocyte is clearly visible in the form of a fine line, which extends across the concavity of the crescent. The black markings in the middle of the crescent represent the pigment.

Fig. 23: Crescent in transition to sphere-form. Pigment in the centre.

Fig. 24: Spheres of the tropical parasite (bone-marrow preparation). Arrangement of the pigment similar to that of the crescents.

Figs. 25-32 are tertian parasites, stained by Romanowsky's method to show the development of the chromatin.

Fig. 25: Small tropical ring.

Fig. 26: Small tertian rings. Double infection.

Fig. 27: Large tertian ring, about twenty-four hours old, with the stippling characteristic of the tertian parasites.

Fig. 28: Half-grown tertian parasite, so-called amœboid form, about twenty-four hours old. Pigment in isolated fine black dots. The massive black grain is the chromatin.

Fig. 29: Commencement of the fission of the chromatin. The seven large black spots are the chromatin on the point of division, the small black dots are the pigment which is still scattered at this stage.

Fig. 30: Fission-form. At its side a normal erythrocyte to show the relative size. The large black dot in the middle is the pigment. The small, more or less distinct black spots (sixteen) are the chromatin granules of the young parasites.

Fig. 31: Male gamete in an erythrocyte. The broad black ribbon is the chromatiu. The protoplasm is scarcely stained, and therefore shown in the photograph merely by the pigment.

Fig. 32: Female gamete. The narrow black stripe at the base is the chromatin. The protoplasm is rather dark, and everywhere contains pigment.

Figs. 33 and 34: Tertian parasites, torn on account of bad preparation, which were only recognisable by the pigmentary stippling. In the figure, however, the pigment is not clearly visible.

Fig. 35: Circular vacuoles in erythrocytes, which might be mistaken for ring-shaped parasites.

Fig. 36: Discoid vacuoles in erythrocytes, which might give rise to confusion.

Figs. 37-43 and Fig. 49: Course of development of the malaria of birds.

Fig. 37: Proteosoma, half-grown, at once recognisable as such by the

displacement of the nucleus of the erythrocyte (sparrow's blood). The dark longish dot is the chromatin.

Fig. 38: Proteosoma, commencing division of the chromatin (sparrow's blood). The black dots which lie at the periphery are the chromatin. The black spot in the middle, with the two connected black specks, is the contracted pigment.

Fig. 39: Fertilized female gamete (zygote) of proteosoma from the contents of the stomach of *Culex pipiens* (Van der Wulp). The black spot is the chromatin (after R. Koch).

Figs. 40-42: Development of the parasite of malaria of birds into vermicular form (after R. Koch).

Fig. 40: A stage of the development of halteridium. The large black spot is the chromatin. The small black granules are the pigment.

Fig. 41: A stage of development of proteosoma. The large black spot is the chromatin. The small black granules are the pigment.

Fig. 42: A vermicular body of halteridium. The large black spot is the chromatin. The small black granules are pigment.

Here the stages of development of the two genuine malarial parasites of birds have been represented to show that in both the commencement of the exogenous stage takes a quite similar course.

Fig. 43: Smallest form of proteosoma cysts on the outside wall of the stomach of *Culex pipiens* (Van der Wulp). Magnified 500 diameters. About half the size of an erythrocyte, and only recognisable as a cyst (zygote) by the grain of pigment.

The Figs. 44-48 of other known blood parasites are added here for the purpose of comparison.

Figs. 44 and 45: Blood parasites of East African apes.

Fig. 46: Parasite of the Texas fever of the ox (*Pirosoma bigeminum*).

Figs. 47 and 48: Blood parasites of the frog (dactylosoma). Fission-forms.*

Fig. 49: Sporozoites of proteosoma. Preparation in glycerine. The sporozoites are shrunken. Magnified 500 diameters.

Fig. 50: Salivary gland of female *Anopheles maculipennis* (Meigen). Magnified 25 diameters. The excretory ducts of the individual gland-lobes are distinctly recognisable. They lead into the common excretory duct. Fresh preparation.

Fig. 51: Flagellated body of the tropical parasite. One flagellum has already separated. Magnified 1,000 diameters. (After R. Koch).

Plate II.

Fig. 1: Female *Culex annulatus*. Magnified 4 diameters (*cf.* p. 25).

Fig. 2: Male *Culex annulatus*. Magnified 4 diameters (*cf.* p. 25).

Fig. 3: Female *Culex pipiens* (Van der Wulp). Magnified 4 diameters (*cf.* pp. 20 and 24).

* The photographs 7-11, 13-18, and 44-48 I owe to the kindness of Privy Councillor Koch.

PLATE II.

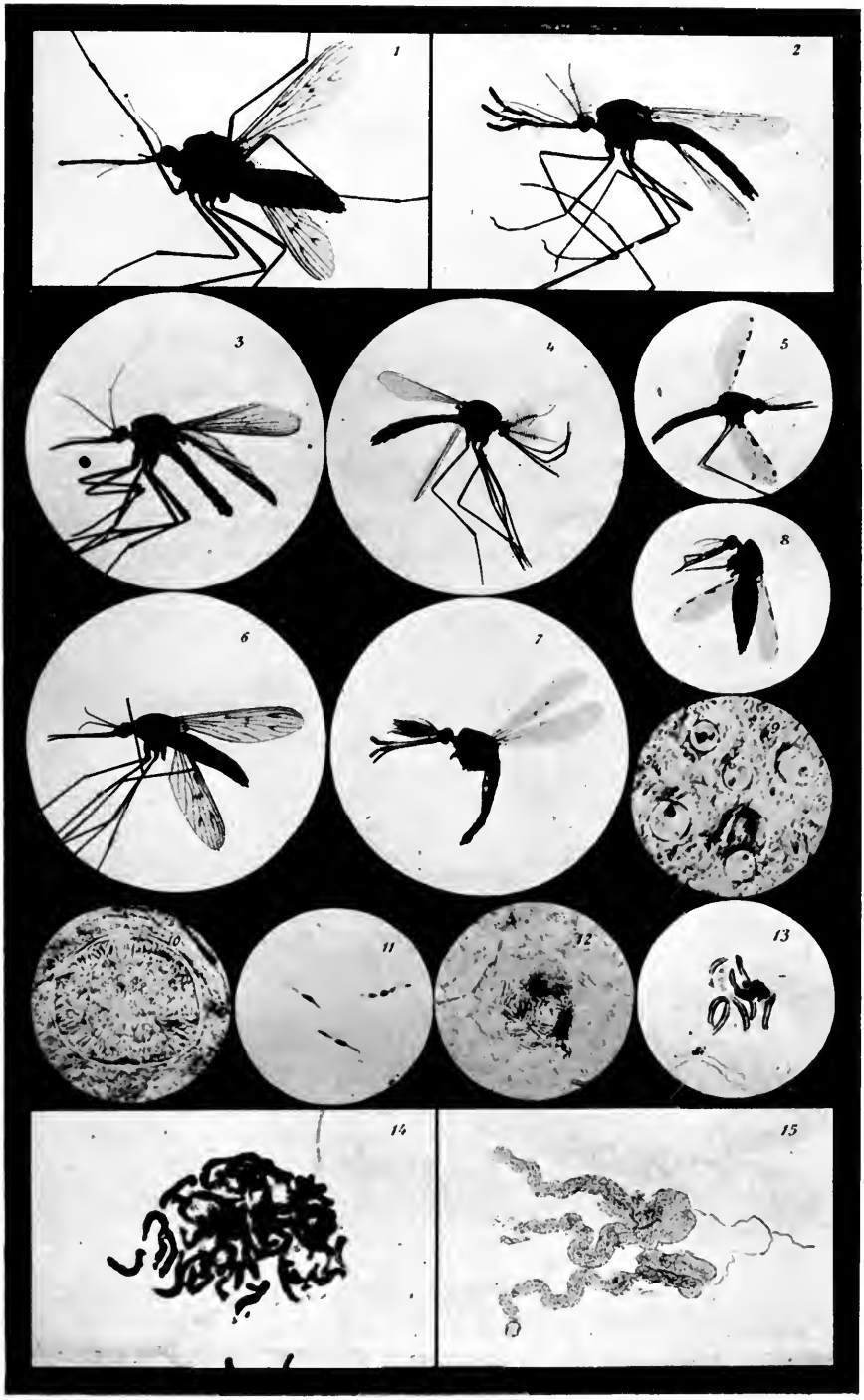

Fig. 4: Male *Culex pipiens*. Magnified 4 diameters (*cf.* pp. 20 and 24).

Fig. 5: Female anopheles (Cameroons). Magnified 4 diameters (*cf.* p. 21).

Fig. 6: *Anopheles maculipennis* (Meigen), female. Magnified 4 diameters (*cf.* p. 21).

Fig. 7: *Anopheles maculipennis* (Meigen), male. Magnified 4 diameters.

Fig. 8: Female anopheles (coast of Zanzibar). Magnified 4 diameters. The abdomen is so thick because the mosquito had sucked so much blood.

Fig. 9: Small cysts of German proteosoma on the external surface of the wall of the stomach of *Culex pipiens* (Van der Wulp). Magnified 500 diameters. Fresh preparation. In the small inner spheres the pigment lies imbedded. These are by no means daughter spheres.

Fig. 10: Cyst of German proteosoma filled with sporozoites. Magnified 500 diameters. Fresh preparation.

Fig. 11: Sporozoites of German proteosoma stained by Romanowsky's method. The black grain lying in the centre is the chromatin. Magnified 1,000 diameters.

Fig. 12: Brown sporozoites. Magnified 500 diameters (*cf.* p. 37). Preparation in glycerine. The margin of the cyst is clearly recognisable. The sporozoites are just on the point of extrusion.

Fig. 13: Transition-forms between brown sporozoites and Ross's spores. Magnified 500 diameters. Preparation in glycerine.

Fig. 14: Ross's spores (black spores). Magnified 500 diameters. Preparation in glycerine.

Fig. 15: The two connected salivary glands, with their common excretory duct, of the female *Culex pipiens* (Van der Wulp). Magnified 50 diameters. The upper gland is complete. The large middle lobe lies deeper than the two side lobes, and appears, therefore, light grey. One of the side lobes of the lower gland is torn off in the preparation, and the middle lobe is folded back upwards. It appears, therefore, as a dark, sausage-like body at the lower margin of the right-hand end of the side lobe.

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